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The Biogenic Transformation of Fine Sediments in Lowland Permeable Catchments

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Abstract

Chalk streams drain areas of outcropping chalk and receive a significant proportion of their discharge from the substantial underground aquifers found within chalk geological formations. Chalk streams flow through open countryside, have high nutrient status, clear waters and a stable hydrological regime and these conditions promote the development of a substantial macrophyte community typically dominated by *Ranunculus* spp. These streams also support large populations of blackfly larvae which are found attached to the surfaces of submerged macrophytes. The larvae feed by using paired cephalic head fans to intercept particles passing in the water column. Blackfly larvae have a low assimilation efficiency and therefore a significant proportion of the ingested material is egested as faecal pellets. Studies have revealed these faecal pellets to be the dominant particles in the suspended load in some systems and this has important implications for particle transport and fate.

This study investigated how blackfly larvae alter the size range of particles within streams through the production of faecal pellets and the presence and temporal dynamics of these faecal pellets within two chalk streams in Southern England. Experiments also established the factors that determine the fate of blackfly faecal pellets by investigating; the interaction between the annual growth cycle of macrophytes and the accumulation and transport of faecal pellets; the controls on the transport of faecal pellets, relating to physical variations in the pellet itself; and the factors that influence the decomposition of this material within the stream.

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1 Introduction

1.1 The physical nature of chalks streams

Chalk streams drain areas of outcropping chalk and receive a significant proportion of their discharge from the substantial underground aquifers found within chalk geological formations. The distribution of chalk rivers within the United Kingdom naturally follows that of the chalk geology and ranges from Dorset on the south coast and runs in an easterly band as far north as Yorkshire (Environment Agency & English Nature, 2004). The physical nature of chalk river systems is strongly influenced by the underlying geology. Chalk has a porosity of around 40 %, therefore the majority of rain falling on areas of chalk percolates through the chalk and down to the aquifer. The ability of the chalk to absorb much of the rainfall creates little surface runoff, causing these streams to have a low stream order and network density (Berrie, 1992). The headwaters of chalk streams occur where the water level within the aquifer intersects with the ground surface, forming springs. For this reason, the headwaters are typically situated relatively low down in the catchment and migrate further down the catchment as the level of the aquifer decreases over the summer. Following recharge of the aquifer, with the autumnal and winter rains, the aquifer level rises, leading to an increase of headwater height, these transient reaches are commonly known as winterbournes (English Nature & Environment Agency, 1999).

Chalk stream hydrology is determined by the underlying aquifer, which in turn relies on climatic conditions. When the aquifers are at their maximum level they contribute to a significant proportion of the stream discharge via springs. At this time chalk streams are at maximum discharge and the winterbournes resume flowing. Stream discharges steadily decrease over the course of the spring and summer, in tandem with the

decreasing aquifer water level, until reaching a seasonal minimum during the late summer. As rain increases in the autumn the aquifers are recharged with a resultant increase in the river levels (Westlake et al., 1972). The influence of the chalk aquifer on chalk streams creates a stable flow regime and smaller peak flows in comparison with other river systems. This is reflected in the ratio of maximum to minimum daily mean flows which can be as low as 3:1 in chalk streams compared to >100:1 for clay catchments (English Nature & Environment Agency, 1999). The hydrology coupled with high width: depth ratios, caused by un-cohesive bank material, gives these systems a low stream power and prevents them from mobilising their gravel beds (Sear et al., 1999). In addition to their low stream power, the minor contribution of surface flows results in low inputs of terrestrial particles and the short stream length limits the downstream export of particles giving chalk streams their characteristically clear waters.

Coupled with their characteristically stable hydrological regimes, chalk streams are noted for their stable thermal regimes (Sear et al., 1999). Groundwater emerges from springs at a steady temperature of around 11°C, and this is reflected in the annual temperature regimes of chalk streams, which relative to rivers fed from surface runoff, are warmer in the winter and cooler in the summer (Berrie, 1992). Small streams located close to springs will be much closer to the ambient spring temperature, perhaps varying by 0.5°C over the year (Westlake et al., 1972), while larger rivers with more diverse water sources, show a wider range of temperatures between 5 - 17°C (English Nature & Environment Agency, 1999), with an annual mean ranging between 10.4 – 10.8°C (Dawson, 1976b).

The distinctive physical environment of chalk streams, coupled with the high degree of anthropogenic influence on the surrounding landscape, places them at odds with river

system definitions such as the River Continuum Concept (RCC). The RCC predicts that the headwaters of a stream will be shaded by riparian vegetation resulting in organic matter inputs dominated by allochthonous detritus. As streams flow down the catchment their physical characteristics, such as width, depth and discharge, increase and organic matter inputs switch to autochthonous production as shading of the channel by riparian vegetation is reduced. Eventually, as the river continues to flow downstream and expand and deepen, the cumulative increase in particles transported from upstream reaches and tributaries increases light attenuation and the river reverts to allochthonous energy sources imported from upstream reaches. Such river systems will only have a community of macrophytes and the associated fauna dominate in the middle, autochthonous, reaches of the river (Vannote et al., 1980).

In contrast to rivers with forested catchments, chalk streams are typically autochthonous throughout their length. They emerge low down into cleared catchments and so are immediately capable of supporting high levels of primary production. The high contribution of aquifer water to the stream's discharge, coupled with little surface runoff and the relatively short stream lengths, reduces the accumulation of particles in the downstream direction. This prevents the river from reverting to a heterotrophic system in the downstream reaches. Thus chalk streams, in common with other small lowland, macrophyte-dominated streams, such as those found in Denmark, are not long enough to experience the predictable longitudinal changes in physical properties that are the dominant control on the development of the RCC predictions (Riis et al., 2001). The RCC predictions were formulated to apply to unmodified systems. However, even in an undisturbed state chalk streams would be unlikely to fulfil the RCC criteria as they would have flowed through lowland woodlands (see below) thereby never developing the extensive autochthonous reaches predicted by the RCC.

Chalk streams originally flowed through lowland woodland and had heavily shaded channels. Under these pristine conditions the majority of macrophytes were refugia species, existing only where breaks in the riparian canopy allowed in enough sunlight to promote growth (English Nature & Environment Agency, 1999). The clearance of the riparian woodland, commencing in Roman times (Environment Agency & English Nature, 2004) switched the dominant energy sources to the stream from allochthonous to autochthonous. The removal of the riparian trees reduces inputs of allochthonous material, notably leaf litter (Baldy et al., 1995) and increases the solar radiation reaching the channel thereby increasing autochthonous production (Horvath, 2004). For streams which are naturally heavily shaded by riparian vegetation the clearance of this vegetation is likely to be the most important anthropogenic activity to impact on the functioning of the stream system (Harrison & Harris, 2002).

1.2 Biology of *Ranunculus* and Simuliidae

Chalk streams flow through open countryside, have high nutrient status, clear waters and a stable hydrological regime and these conditions promote the development of a substantial macrophyte community. Within the Rivers Frome and Piddle the dominant submerged macrophyte is *Ranunculus penicillatus* var. *calcareus* (R. W. Butcher) C. D. K. Cook (hereafter referred to as *Ranunculus*) (Westlake et al., 1972). *Ranunculus* is an herbaceous perennial found in the fast flowing reaches of streams containing a stable substratum. The stems grow to a length of up to 4 m and are orientated in the direction of the flowing water giving the plant a distinctive teardrop shape with the tapered point at the downstream end of the plant (Dawson, 1976b).

A key determinant on the peak biomass of *Ranunculus* within a given year is discharge, with a positive relationship seen between mean discharge and the areal extent of *Ranunculus* in March to May (Wright et al., 2002). The initiation and rate of growth is determined by water temperature and therefore is influenced by the distance of the stands from springs and the ambient air temperature (Westlake et al., 1972). Dawson (1976b) described the annual *Ranunculus* growth cycle as undergoing four distinct phases. The extension phase from late winter to around April is a period of rapid increase in plant biomass leading to extensive coverage of the stream surface. This is followed by a consolidation phase during which, although the areal extent of *Ranunculus* does not increase greatly, the plants continue to increase in biomass and flowering and seed production occurs. During flowering the main plant stems thicken in size, become hollow and float to the surface at which point the *Ranunculus* occupies a significant proportion of the channel. The timing of flowering varies within the river and the peak flowering period near the springs can occur two months before peak flowering at the furthermost downstream site due to the higher temperature of the water

near the springs early in the growth cycle. The timing of flowering dictates the initiation of the decline phase of the *Ranunculus*, in which plant biomass declines, commencing around one month post-flowering.

By late summer the *Ranunculus* biomass has significantly declined and the remaining stands can become overgrown by *Rorippa nasturtium-aquaticum* (L) Hayek var. *siifolium* and *Apium nodiflorum* (L.) Lag. (Westlake et al., 1972). These species are broad-leaved emergents and are found in the slower-flowing sections of the river such as the channel margins and areas of slow flow created by *Ranunculus* stands. These species are less hardy than *Ranunculus* and are largely lost from the system during the colder winter months (Gregg & Rose, 1982). The increased discharge during winter removes both senescent plant material and the fine sediments that have accumulated under the stands. This flushing action promotes the regrowth phase of the *Ranunculus* and biomass again begins to increase (Dawson, 1976b).

Aquatic macrophytes have developed morphological adaptations to cope with flowing water. *Ranunculus* are able to tolerate some of the highest water velocities of any macrophytes and have developed highly flexible stems and leaf filaments that bend in the direction of the flow, causing the plant to form a closed, compressed canopy (Sand-Jensen & Mebus, 1996). These adaptations reduce the drag acting upon the plant and divert flows around the plant structure preventing erosion and the uprooting of the stand (Sand-Jensen, 2003). The compressed canopy structure of the *Ranunculus* stands causes variation in the velocities of the stream water. Those flows deflected around and above the stands are accelerated while the velocity of the water that enters the stand is rapidly attenuated by the dense layer of macrophyte tissue and is matched by a similar decrease in turbulence (Sand-Jensen & Pederson, 1999). These diverse flows increase the

available habitat within the river (Hearne & Armitage, 1993) and are associated with increased population densities of salmonids (Sand-Jensen et al., 1989) and macroinvertebrates which shelter from both high flows and fish predation (Gregg & Rose, 1982; Wright et al., 2003). As biomass increases and plants fill the channel during early summer there is an increase in hydraulic drag. This results in increased water depth, being up to four times higher than in *Ranunculus*-free reaches, and decreased water velocity (Dawson, 1978). High water levels lead to flooding of adjacent land necessitating the removal of the macrophytes (Hearne & Armitage, 1993). Weed-cutting is also undertaken on reaches used for fishing to prevent the snagging of lines and to maintain areas of spawning gravels (Wright et al., 2003).

The presence of large macrophyte stands within the water column impacts on the transport of material through the reach. The decline in flow velocity within the stands results in a reduction in the velocity of near-bed flows and the accumulation of extensive deposits of fine inorganic and organic particles (Sand-Jensen et al., 1989; Sand-Jensen & Pederson, 1999). In addition to the low velocities, the extensive root network increases the stability of the substratum by increasing the embeddedness of the stones through binding by the rhizome and roots and this enhances the retention of the deposited particles (Fritz & Feminella, 2003). Low flow velocities within the stand increase the water residence time which, coupled with the increased levels of organic matter, results in denitrification rates three-times higher than those in unvegetated deposits (Sand-Jensen, 1998). This enhanced trapping of fine sediments and their associated nutrients by macrophytes can be of such magnitude as to reduce the downstream loading of estuaries and lakes (Sand-Jensen et al., 1989). Macrophytes also increase the retention of large organic particles within the channel as leaves and other organic materials in transport become snagged on the plants (Brookshire & Dwire,

2003; Horvath, 2004). This promotes the processing of the material by macroinvertebrates and microorganisims again increasing the retention of nutrients and energy within the reach (Prochazka et al., 1991).

Changes to current velocities cause indirect effects such as altering the nutrient status of sediments within macrophyte stands. The nutrient status of the underlying sediments is positively related to the nutrient concentration within the overlying macrophyte tissue, shoot density and overall macrophyte biomass indicating that deposited particles are an important source of nutrients to the plants (Chambers et al., 1991). The tendency of macrophytes to grow as discrete patches creates a chemically heterogeneous substratum with areas of inorganic and nutrient-poor sediments interspaced with organic and nutrient-rich sediments within stands (Clarke & Wharton, 2001). The seasonal pattern of *Ranunculus* growth, varying from little or no plant tissue in the winter to very high summer biomasses, exerts a strong temporal influence on the processes impacted by macrophytes (Clarke, 2002).

Within macrophyte stands, organic-rich fine sediments support a detritivorous fauna (Sand-Jensen et al., 1989), and provide a substratum for extensive autotrophic and heterotrophic epiphytic communities (Sand-Jensen et al., 1989) and diatoms (Gregg & Rose, 1982). Lowland streams with extensive macrophyte communities also support large populations of blackfly larvae which feed by attaching to the surface of macrophytes (Sand-Jensen et al., 1989). Within chalk streams blackfly larval abundance is positively related to the surface area of *Ranunculus* as this increases the availability of attachment sites from where the larvae can feed (Ladle et al., 1972; Wright et al., 2003).

Within both the Rivers Frome and Piddle the blackfly community is dominated by *Simulium* (*Wilhelmina*) *equinum* L. and *Simulium ornatum* Meigen (Ladle et al., 1972;

Ladle et al., 1977; Bass, 1998). An extensive study on the River Frome found the following species; *S. erythrocephalum* De Geer, *S. lineatum* Meigen, *S.* (*Eusimulium*) *aureum* Fries, *S. vernum* Macquart and *S. trifasciatum* Curtis, previously *S. spinosum* (Ladle et al., 1977). There has been no marked shift in the composition of the blackfly community or their distribution in the Rivers Frome and Piddle since the studies described above were conducted (J. Bass pers comm.).

Blackfly larvae influence a number of processes within streams. Larvae are a food source and are an important link in many stream foodwebs as they are consumed by both vertebrates, such as fish and birds (Malmqvist et al., 2004), and by invertebrate predators (Yule, 1996). This impacts on trophic dynamics as the larvae act as a pathway by which ingested microbial carbon is transformed into larval biomass and then transferred to higher vertebrate trophic levels through predation (Parkes et al., 2004). Attention has also focused on the role of larvae on the clearance of material from the water column. Morin et al (1998) investigated the impact of a blackfly larval aggregation on the loss of seston from a lake outlet river and found that the larvae were capable of removing 0.8 –1.4 % of the stream seston per linear metre of stream length. Other studies have recorded removal rates of 0.3 % per metre (Parkes et al., 2004) and demonstrated the increased retention of particles when larvae are present (Wotton et al., 1996). Studies tracing the loss of fluorescently labelled bacteria from the water column found blackfly larvae to have a measurable impact on the loss of the bacterial cells, although this was low compared to losses through physical processes. However, the study stream had a low population density of larvae of 4,800 larvae m^{-2} (Hall et al., 1996), several orders of magnitude below that found in chalk streams (Ladle et al., 1972).

Within rivers, larvae typically inhabit fast flowing environments such as rapids and riffles (Morin & Peters, 1988; Malmqvist et al., 2001); geomorphological features that are rarely found in chalk streams. Within chalk streams the leaves and stems of *Ranunculus* stands are the favoured environment as flows are deflected and accelerated around the plants creating an environment analogous to fast-flowing geomorpholgical features (Ladle et al., 1972). Once they find a suitable surface the blackfly larvae anchor themselves to the surface of the *Ranunculus* to prevent themselves being washed downstream. They attach to the *Ranunculus* plant by depositing a silk pad on to the plant surface and secure themselves in place by inserting a ring of hooks that surround the abdominal proleg into the silk pad (Crosskey, 1990).

Once anchored to a suitable surface larvae commence feeding by filtering particles from the water column (Crosskey, 1990; Malmqvist et al., 2004). Particles are collected by paired cephalic head fans which intercept any particles that pass the larvae in the water column (Crosskey, 1990). Larvae are capable of ingesting material of a wide range of particle sizes, from dissolved organic matter (particles <0.45 μm) to particles 350 μm in diameter (Miller et al., 1998). Although larvae non-selectively collect particles, they demonstrate differences in the capture efficiency for particles of different sizes in relation to the larval size (Wotton, 1984). This non-selective feeding strategy results in larval diet being dependent on the dominant particles within the system. Thus larvae living in lake-outlet streams have a diet dominated by phytoplankton (Morin & Peters, 1988; Parkes et al., 2004) while larvae in snowmelt rivers have a diet rich in inorganic particles that are washed into the river following the spring thaw (Malmqvist et al., 2001). The larvae ingesting inorganic particles receive their energy from organic materials associated with the particles (Wotton, 1978; Parkes et al., 2004).

Blackfly larvae have a relatively low assimilation efficiency, as low as 2 % (Wotton, 1978). Thus, from a qualitative aspect, a significant proportion of the ingested material is egested, as faecal pellets, relatively unchanged. Although essentially unmodified qualitatively, egested material is substantially modified in its physical appearance. The faecal pellets are a matrix of fine particles tightly bound into a distinctive, smooth-surfaced, ellipto-cylindrical form (Ladle et al., 1987; Malmqvist et al., 2001). Blackfly larvae have a gut throughput time of between 20 – 30 mins (Ladle et al., 1972; Miller et al., 1998) up to 1 h (Wotton, 1978) resulting in the production of large numbers of faecal pellets. The mean number of pellets produced per larva has been recorded at between 575 (Wotton et al., 1998) to 737 pellets day^{-1} (Malmqvist et al., 2001). Studies have revealed faecal pellets to be the dominant particles in the suspended load in both large boreal rivers and lake-outlet streams (Wotton et al., 1998; Malmqvist & Wotton, 2002), forming up to 30 % of the suspended load by mass (Malmqvist et al., 2001; Malmqvist & Wotton, 2002). Comparisons of faecal pellet flux through a large Swedish catchment found a positive relationship with stream size and an increase in faecal pellet concentration with distance downstream from the source. There was also a positive relationship between faecal pellet flux and the location of lakes; this was attributed to the presence of lake outlets, a favoured larval habitat due to the high quality of the food resources draining from the lake (Malmqvist & Wotton, 2002).

Blackfly larvae are capable of living at very high population densities with over 1.0 x 10^6 larvae m^{-2} recorded on the weir lip of a lake outlet (Wotton, 1987). The population densities of chalk streams are lower, although still substantial, with up to 300,000 larvae m^{-2} recorded in the Bere Stream (Ladle et al., 1972). These high population densities coupled with the large numbers of faecal pellets produced per individual larva means that larval aggregations process and repackage huge quantities of material into faecal

pellets which are released back into the stream. The impacts of larvae on the suspended load of the river has been shown to be highest when discharge is low and larval populations at their greatest densities, as there is increased likelihood of particles being intercepted by the larvae (Wotton et al., 1996; Morin et al., 1998). Therefore within chalk streams the larvae are expected to have their greatest influence on suspended material during the summer months as peak larval abundance coincides with both low discharges and low suspended particle concentrations so increases the likelihood of interception by the larvae (Ladle et al., 1972). Extrapolations from the quantity of material ingested per larva multiplied by the population density suggest that, at peak larval density and lowest suspended particle concentrations, larvae are capable of removing the entire suspended load of a chalk stream within 600 m (Ladle et al., 1972).

It has long been recognised that the faeces of filter-feeding invertebrates have important implications for stream ecology. The combination of capture from the water column, low assimilation efficiency and egestion back into the stream impacts on the availability of important detritus resources for other components of the stream community (Wallace et al., 1977). Hershey et al. (1996) found that a dense aggregation of blackfly larvae increased both the quantity and size of particulates below the aggregation as a result of the larvae ingesting dissolved and particulate organic matter, binding this material into much larger faecal pellets, and egesting these back into the stream. The production of faecal pellets, which are much larger in size than their constituent particles, has important implications for fine sediment dynamics as large particles sink at a faster rate than small particles (Wotton et al., 1998), and this promotes increased retention within the system. The retained material is then available to the rest of the stream community and represents a valuable resource for the system. Wotton et al. (2003) calculated the quantity of carbon incorporated into larval biomass compared to that egested by the

larvae and showed that larvae egest 68 times more carbon than they incorporate into their biomass. This suggests that, at the ecosystem level, the cycling of carbon through larval faecal pellets may be a more significant process then the incorporation of carbon into larval biomass.

The aggregation of particles in freshwater systems, such as occurs during the formation of faecal pellets, has important implications for particle transport and fate, as the constituent particles of the aggregation may settle in areas that would not have been suitable prior to aggregation (Droppo et al., 1997). Depositional areas within rivers promote the settling of blackfly faecal pellets. The huge quantities of faecal pellets transported by large rivers are deposited in areas with reduced flow such as river margins, dead water zones and estuaries (Malmqvist & Wotton, 2002), while within chalk streams, macroinvertebrate faecal pellets contribute a significant proportion of the organic material deposited on the substratum (Ladle & Griffiths, 1980). These biodeposits can be quantitatively significant relative to other organic matter processes. Studies on a lake-outlet stream showed that faecal pellet deposits on the stream substratum were of a similar order to the leaf litter inputs to the stream and were therefore an important component of the organic matter dynamics within these streams (Wotton et al., 1998). The retention of faecal pellets within river systems promotes increased utilisation of the material contained within the pellets by the stream community (Yule, 1996). The impact on ecosystems may even extend beyond the confines of the stream channel. It has been hypothesised that the transfer of faecal pellets from the water column onto river banks during flood events may fertilise the surrounding vegetation through the release of organic carbon and nitrogen bound within the pellets. This is a possible explanation for the traditional farming practises of flooding land adjacent to rivers to increase fertility (Malmqvist et al., 2004).

1.3 Organic matter processing in streams

The abundance and processing of fine particulate organic matter is of great significance to the functioning of stream ecosystems. In many systems it is the primary food source for macroinvertebrate consumers and so underpins many lotic foodwebs (Hershey et al., 1996; Miller et al., 1998). Measures of ecosystem respiration are related to the degree and timing of organic matter inputs to streams showing these to represent a significant source of energy (Acuna et al., 2004). Organic matter within streams is colonised by microorganisms that utilise the material as their principal energy source. The associated microbial biomass is subsequently ingested by consumers, transferring energy to higher trophic levels (Meyer, 1994; Schlickeisen et al., 2003). Much of the work on organic matter processing in lotic systems has focused on the processing of leaves as these are predicted to represent a significant source of energy for rivers that are surrounded by extensive riparian vegetation (Vannote et al., 1980; Baldy et al., 1995).

The breakdown of leaf material within streams is dominated by biological processes, particularly shredder macroinvertebrates, fungi and bacteria, as opposed to physical processes (Baldy et al., 1995). Initially, decomposition, when leaf tissue is intact and contains the complex structures that make them resistant to breakdown, is mediated by fungi. Fungi process leaves with the aid of invasive vegetative hyphae that produce digestive enzymes capable of breaking down the lignin structures (Schlickeisen et al., 2003). The role of bacteria increases later in the process as the leaves become increasingly conditioned and fungal biomass begins to decline (Baldy et al., 1995). Bacteria play a greater role in the decomposition of plants that do not contain the lignin structures found in terrestrial and emergent species such as submerged and floating macrophytes (Schlickeisen et al., 2003), while submerged seagrass leaves are decomposed by bacteria with little or no contribution from fungus (Newell, 1981).

Terrestrial leaves do not provide the dominant energy source in chalk streams due to the lack of riparian trees and the low ratio of minimum to maximum flows, which reduces flooding and limits the wash-in of leaves (Dawson, 1976a). Dawson (1976a) constructed a speculative organic matter budget for a chalk stream in Dorset and calculated that leaf litter inputs contributed between 2 – 3 m. tonne organic matter yr^{-1}, while macrophytes contributed 13 m. tonne organic matter yr^{-1} and algae another 6. Contemporary macrophyte-rich chalk streams have a different temporal input of organic matter than pristine chalk streams. The seasonal nature of leaf litter inputs results in a strong pulse that dominates the energy inputs to the system while the seasonally closed canopy of the riparian trees reduces photosynthetically available radiation to the channel by 80 % leading to a decrease in primary production (Acuna et al., 2004). In contrast, aquatic rooted macrophytes provide a more consistent source of organic matter than terrestrial leaves as they persist within the stream throughout much of the year.

As chalk streams support high population densities of blackfly larvae the resultant benthic deposition of faecal pellets in the stream (Ladle & Griffiths, 1980; Dawson, 1981) provide an alternative source of organic material. Much of the processing of these biodeposits will be dominated by microorganisms (Wanner & Pusch, 2001). Benthic organic deposits play a key role in the supply of dissolved organic matter to the water column in New Zealand macrophyte-dominated streams (Wilcock & Croker, 2004) which are similar to chalk streams. Furthermore, particles deposited on the uppermost layers of the sediments will have a disproportionately greater influence on stream processes as they are exposed to high oxygen concentrations from the overlying water column, will be more labile and will have a greater chance of being utilised by stream consumers (Cushing et al., 1993). The processes that lead to benthic organic deposits are arguably more important to river ecosystem processes than the quantity of organic

inputs to the stream. Retained material is utilised by the stream community while the material that passes through the system contributes little as it is not extensively utilised (Prochazka et al., 1991; Acuna et al., 2004).

Within lowland macrophyte dominated streams the trapping of organic matter by physical structures such as macrophytes and filtration by bed sediments (Wanner & Pusch, 2001) leads to areas of organically enriched sediments that are sites of intense microbial processing. Filter-feeding macroinvertebrates further increase retention as fine organic material in transport is intercepted, egested as faeces and transferred to the substratum for consumption by benthic invertebrates (Cushing et al., 1993; Yule, 1996). The strongly seasonal influence of discharge and macrophyte growth faced by temperate streams leads to the removal of organic deposits as the features that initially promoted their deposition, low discharge and macrophytes, are not present over the autumn and winter. Although determining the factors that promote the removal of organic particles - resuspension, transport and loss of material from the reach - is clearly of great importance to stream processes this area has received relatively little attention to date (Cushing et al., 1993).

1.4 Project aims

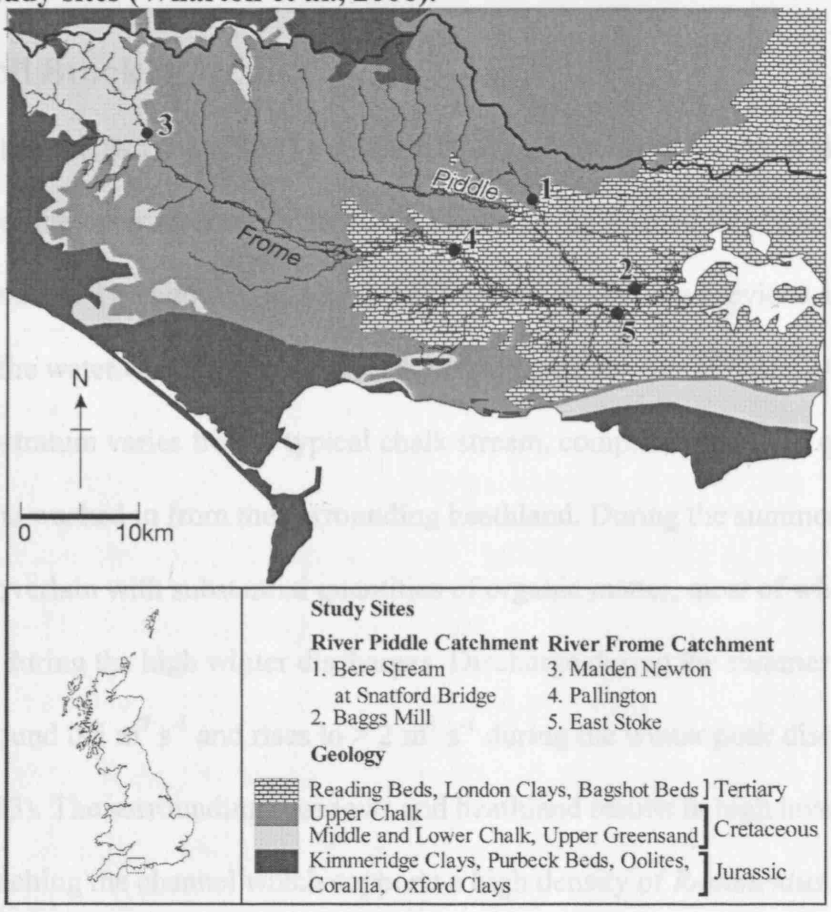
This study aims to quantify the presence and temporal dynamics of blackfly larvae faecal pellets within chalk streams. Experiments will aim to establish the factors that determine the fate of blackfly faecal pellets. These include controls on the transport of faecal pellets, relating to variations in the pellet itself and that of the stream environment, to the factors that influence the decomposition of this material. To provide answers to these issues the following questions will be asked;

1. What is the annual variation in the abundance and biomass of faecal pellets along the Rivers Frome and Piddle?
2. Do blackfly larvae alter the size range of particles within the stream and what factors determine the retention of their faecal pellets?
3. How does the stream environment impact on the transport of faecal pellets? Specifically, what is the interaction between the annual growth cycle of macrophytes and associated fine sediments and the accumulation and transport of faecal pellets through a reach?
4. How are faecal pellets bound together and what factors influence their degradation?

2 Description of field sites

A total of six main sites, located on three rivers were used for this study; two sites on the River Piddle, three on the River Frome, both in Dorset and one on the River Chess, Bedfordshire. Different sites were selected according to their suitability for the research being undertaken and the practicalities of conducting the research. The sites selected for each section of the study are listed the respective chapters.

Figure 2.1; Geological map of the Frome, Piddle catchment showing location of the principle study sites (Wharton et al., 2006).



2.1 Maiden Newton

An upstream sampling site on the River Frome was selected at Maiden Newton (SY 595983) (Figure 2.1). The sampling site is downstream of a railway bridge and comprises of a wide, shallow stream section with low sinuosity. The stream runs adjacent to domestic gardens on one bank and a disused water meadow on the other

bank. The water meadow is intersected with a number of narrow channels, fed predominantly by springs although during high discharge the main channel feeds into these. The main channel has a mean width of 7.0 m, mean depth of 0.22 m and a mean discharge of 0.6 $m^3 s^{-1}$(Wharton et al., 2006). The channel substratum consists of coarse, flinty gravels, interspaced with localised coarse sand deposits. The macrophyte community is dominated by abundant *Ranunculus* growth, although areas with excessive riparian shading preclude the *Ranunculus* growth in places. There was very little growth of either *Rorippa nasturtium-aquaticum* or *Apium nodiflorum*.

2.2 Tadnoll Brook

The Tadnoll Brook (SY 792875) is a small tributary of the River Frome. It is distinct from many of the other streams on the River Frome as the surrounding land consists of heathland and flood meadows. Despite the differences in land use previous studies have shown that the water chemistry is similar to typical chalk streams (Pinder, 1983). The channel substratum varies from a typical chalk stream, comprising of large quantities of sand deposits washed in from the surrounding heathland. During the summer months the sand is overlain with substantial quantities of organic matter, most of which is washed out during the high winter discharges. Discharge during the summer months is typically around 0.5 $m^3 s^{-1}$ and rises to > 2 $m^3 s^{-1}$ during the winter peak discharges (Pinder, 1983). The surrounding meadows and heathland results in high levels of solar radiation reaching the channel which supports a high density of *Ranunculus*.

2.3 East Stoke

The downstream sampling reach on the River Frome was located at East Stoke (SY 870869) (Figure 2.1). The sampling site was on a wide meander bend, 16.7 m wide, the mean depth was 0.38 m and the discharge was 5.5 $m^3 s^{-1}$ with a substratum consists of

coarse flinty gravels (Wharton et al., 2006). The bank on the outer curve of the meander bend is relatively high, up to 1m above the stream surface and is being actively eroded. Flows on the inside of the meander bend were much slower and these conditions promoted some localised deposits of fine material.

The surrounding land is used for grazing cattle which maintains a low grass sward, resulting in minimal channel shading from riparian vegetation. These conditions, coupled with the fast flows at this site, promote substantial *Ranunculus* growth although the slow flows on the inside edge of the meander bend allow some limited growth of *Rorippa nasturtium-aquaticum* and *Apium nodiflorum*.

2.4 Bere Stream

The uppermost site on the River Piddle was located at Snatford Bridge (SY 856930) (Figure 2.1) on the Bere Stream. The Bere Stream is a tributary of the River Piddle and the sampling site is located 1.75 km from the confluence of the two rivers. The channel substratum is comprised of flinty gravels with no substantial deposits of sand, in contrast to Maiden Newton and Tadnoll Brook. The channel has a mean width of 6.7 m, a mean depth of 0.29 m and mean discharge of 0.7 m^3 s^{-1}(Wharton et al., 2006). The upstream limit of the sampling reach was delineated by the presence of large trees which heavily shaded the channel and prevented macrophyte growth, after this point the channel flows through agricultural land for approximately 150 m before entering a wood. Once within the wood the heavy shading of the channel precludes any macrophyte growth. Bere Stream showed the greatest variation in the macrophyte community of all of the sites used in this study. During winter, the macrophytes occur at low abundance with only limited patches of *Ranunculus* present, these patches expand and dominate the channel early in the season. As summer progresses the channel

gradually becomes dominated by *Apium nodiflorum* and *Rorippa nasturtium-aquaticum* which almost completely cover the channel in time.

2.5 Baggs Mill

The lower reach on the River Piddle was located 6.25km from Snatford Bridge at Baggs Mill (SY 912876) (Figure 2.1) just upstream from the town of Wareham. The channel has a mean width of 7.7 m, mean depth of 0.32 m and a mean discharge of 2.6 m^3 s^{-1}(Wharton et al., 2006). The channel is heavily modified; the reach upstream flows under a railway bridge and is constrained within a heavily engineered channel. There is a weir approximately 60m downstream of the sampling site and this prevents any tidal influence on the sampling reach. The substratum is finer then than that found at many of the other sites with extensive sand deposits contained within the channel. The flow is deep and relatively fast, with modest *Ranunculus* growth present in the centre of the channel. Where flows are retarded along the edges of the channel there is substantial growth of *Apium nodiflorum* and *Rorippa nasturtium-aquaticum.*

2.6 River Chess

The River Chess (TQ 004986) is located in Bedfordshire, it is a small Chalk Stream which rises on the Chiltern Downs near Chesham and flows through agricultural land before joining the Rivers Gade and Colne. The channel has a mean width of 9.7 m, a mean depth of 0.21 m and a mean discharge of 0.2 m^3 s^{-1} (P. Joyce, pers. comm.). The channel substratum is comprised of large flinty gravels which become covered in a thick layer of organic material during the summer as the flows decrease. *Ranunculus* is present within the deepest and fastest flowing reaches of the channel although the dominant macrophytes are *Apium nodiflorum* and *Rorippa nasturtium-aquaticum.*

3 The abundance and biomass of faecal pellets within chalk streams

3.1 Introduction

Blackfly larvae can remove between 0.8 – 1.4% of the suspended stream seston per longitudinal meter of stream (Morin et al., 1998). The larvae ingest small particles and then egest these as much larger faecal pellets; these consist of an aggregate of tightly bound, organic and inorganic particles (Hershey et al., 1996). The interception and processing of stream seston by blackfly larvae has important implications for stream ecosystems.

Blackfly larvae have an assimilation rate as low as 2% (Wotton, 1978), thus material ingested by larvae will not be lost to the stream. It will be egested in an essentially unmodified form, except for size, and is still available to the system. The excreted material behaves in the stream environment in a different manner to the original, unmodified seston (see Chapter 50 and this impacts on the flux of material and its eventual fate within the stream (Monaghan et al., 2001).

Studies from other river systems, found that the species of larvae living in the Frome / Piddle catchment inhabit areas of flowing water, typically between 0.5 – 1.2 m s^{-1} (Crosskey, 1990), so freshly excreted pellets enter regions of high stream flow and are transported downstream as a component of the stream seston. In large northern rivers over 30% of the stream seston by mass can be in the form of blackfly faecal pellets (Malmqvist & Wotton, 2002).

Under suitable conditions these pellets will settle out of suspension to form faecal pellet deposits on the stream substratum (Ladle & Griffiths, 1980; Wotton et al., 1998). The values for carbon deposited as blackfly faecal pellets reported by Wotton (2.9 g m^{-2} d^{-1})

were similar to those reported for Zebra mussels, *Dreissena polymorpha*, (3 g m^{-2} d^{-1}) (Strayer et al., 1999). This freshwater bivalve has well documented, large-scale impacts on its environment that include declines in plankton communities and changes to the fate of particles due to their high filtration rates both of which lead to changes in both primary producers and secondary consumers as resources within the system are altered (Strayer et al., 1999).

The interception and removal of suspended material and its subsequent transformation into faecal pellets and pseudofaeces by bivalves causes enhanced deposition rates over the passive settling processes that normally occur (Taghon et al., 1984; Ragnarsson & Raffaelli, 1999; Strayer et al., 1999; Norkko et al., 2001; Giles & Pilditch, 2004; Vaughn et al., 2004). Sediment samples taken from the Palos Verdes continental shelf were analysed for both aggregated and disaggregated particle size distribution. Those samples exhibiting the largest differences were dominated by faecal pellets (Drake et al., 2002) as much of the sediment had been aggregated into faecal pellets through invertebrate feeding activity. This demonstrates the role that invertebrate mediated aggregation can play in altering bulk sediment characteristics.

It is expected that there will be large numbers of faecal pellets retained within the Frome and Piddle, as rivers with large populations of suspension feeders have an enhanced ability to retain particles (Wotton et al., 1996), much of this retention will be in the form of faecal pellets. Macrophyte stands in lowland rivers are well known stores of fine sediments (Sand-Jensen, 1998; Wood & Armitage, 1999) and it is likely that these will also act to trap blackfly faecal pellets. Blackflies in the Frome / Piddle catchment are typically found attached to the leaves and stems of *Ranunculus* plants

(Ladle et al., 1972) and therefore it is hypothesised that these macrophytes will act as a trap for significant numbers of pellets.

Much of this deposited material is likely to have been lost from the system if it had not been trapped as faecal pellets and studies have shown that faecal pellet deposits can be of a similar order to other detritus inputs such as allocthonous leaf litter inputs (Malmqvist et al., 2001). A study on a steep tropical stream found that there were few physical structures capable of trapping particles and that the interception and ingestion by filter feeders was the dominant process that retained FPOM within the system and enabled the resource to become available for other benthic invertebrates (Yule, 1996). The fate of organic deposits has an important impact on stream systems as the organic matter content of sediments is an important predictor of macroinvertebrate populations (Wood & Armitage, 1997).

The current study aims to investigate blackfly faecal pellet dynamics in the Frome / Piddle catchment over the course of a year as this has not previously been examined in chalk stream systems. The annual variation in the abundance and size of faecal pellets transported in the water column will be determined. Areas promoting the deposition of faecal pellets, such as *Ranunculus* stands, will be sampled to look at variation in the size and abundance of the faecal pellets. The above data will be used to create estimates of faecal pellet biomass in both transport and as deposits within the system as these may represent a significant process affecting organic matter dynamics within chalk streams.

3.2 Methods

3.2.1 Field collection of samples

To determine annual variation in blackfly faecal pellets in the Frome / Piddle catchment samples were collected between March 2003 and February 2004. No samples were collected in January 2004 as it was thought that the annual faecal pellet dynamics would have ceased by January. Subsequent processing of the samples in December and January showed that there were still substantial numbers present during these months and so a further sample was taken in February to capture the full annual cycle. Sampling was undertaken monthly, typically during the first week of the month. It was felt that monthly samples would provide a suitably fine resolution to the annual dynamics whilst providing the time necessary to process the collected material.

To determine whether there were any differences between the two river systems and within each of the rivers, intensive sampling was undertaken at an upstream and downstream site on both the Rivers Frome and Piddle. On the River Piddle the upstream site was located at Bere Stream and the downstream site at Baggs Mill, while on the Frome the upstream site was at Maiden Newton and the downstream site at East Stoke.

To determine longitudinal differences in abundance through both of the river systems a number of intermediate sites were selected between the two principal sampling sites on each river. Three sites were selected on the River Frome, moving from upstream to downstream these were Frampton (SY624950), Greys Bridge (SY700908) and Woodsford (SY769909). The distance between the two main sampling sites on the River Piddle was less therefore only two intermediate sites were selected. They were at Hyde (SY866906) and Trigon Farm (SY884886).

At each of the intensively sampled sites a representative *Ranunculus* stand was selected for intensive study, it was not possible to select replicate stands at each of the sites, as the time necessary to process the collected samples would have been prohibitive. It was decided that it would be more important to look for inter-reach differences rather then intra-reach differences.

At each of the *Ranunculus* stands two hollow steel stakes were hammered into the stream substratum towards the upstream end of the stand. These pipes enabled a metal frame to be placed over the top of the stand for measurement purposes. The frame consisted of a commercially available builders scaffold 1.4 m x 1 m and was supported by metal rods, 100 cm long and 1 cm diameter placed inside the hollow legs of the scaffold. These were placed into the stakes in the streambed, enabling the framework to be lifted clear of the water surface thus minimising interference with the stream flow.

The placement of the metal frame at a fixed location within the channel enabled measurements of absolute change in *Ranunculus* growth and *Ranunculus* development in relation to other stream features to be recorded. The stand dimensions were measured monthly and the whole reach surveyed using a total station. This recorded the location of the stand and its development, as well as the location and dimensions of adjacent *Ranunculus* stands, other macrophyte species and permanent features of the stream reach.

To determine if the presence of *Ranunculus* stands influenced the abundance of faecal pellets in transport, water samples were collected immediately upstream and downstream of each study stand. Water samples were collected in 500 ml Schott Duran reagent bottle, with a total volume of 610 ml. Trial collections showed that this volume

sample bottle collected enough stream water to determine the abundance and size of the faecal pellets in transport within the river.

The technique for the collection and storage of the suspended material followed that of Wotton et al. (1998) and Malmqvist et al. (2001) To collect the sample the bottles were placed just below the water surface with the bottle aperture pointing upstream until the bottle was fully filled. Collected water samples were passed through a 25 μm Monyl mesh net to filter the suspended particles prior to storage. Monyl mesh was selected for the net as the weave of the net is flat allowing material collected in the net to be easily washed off with none trapped on the mesh. Material retained within the net was washed to the bottom of the net with distilled water using a plastic wash bottle. This filtrate was secured by folding the net over the material and the net inverted and placed over a 60 ml wide mouthed glass bottle. This caused most of the filtrate to fall into the sampling jar and any remaining on the mesh was washed into the jar with a solution containing 70% Industrial Methylated Spirits (IMS) and 30% distilled water. Five replicates were taken at each sampling location.

The intermediate sites were generally hard to access safely, therefore collections were made by sampling from bridges spanning the rivers using a technique similar to that used by Malmqvist et al. (2001). Water samples were collected by lowering a graduated bucket from the bridge into the river, the volume of water collected was noted and the sample treated using the same technique as that used to process the water samples at the main sites (see above).

Faecal pellets deposited within each of the main sampling sites were collected using 37 mm diameter and 30 cm long hollow Perspex piping, the coring end of the tube was bevelled to aid it's insertion into the deposits. Cores were taken from the *Ranunculus*

stand under investigation and from marginal organic deposits. Coring locations within the *Ranunculus* stands were picked using random number tables to generate a set of coordinates. Using the metal scaffold as a reference point the core location was selected using the coordinates, a note was made of each sampling point so that subsequent cores would not be taken at a previously sampled location.

Samples were taken by pushing the Perspex core into the sediment as far as possible, the other hand was pushed into the sediment around the core, worked down to the core base and the bottom of the core covered to prevent the sample being lost as the core was removed. The core was removed from the sediment and a bung inserted in the bottom end of the piping to maintain the integrity of the sampled material.

Faecal pellets are deposited on to the top organic-rich layer of the sediments so all of the organic-rich material was removed from the sample. A p1000 Gilson pipette was inserted into the top of the Perspex core and the organic-rich material drawn into the pipette. This material was placed in 60 ml wide mouth bottles and preserved in 70% IMS. On the occasions when there was only a small quantity of material present and the pipette could not be used, then the contents of the core were transferred to the net and treated in the same way as the suspended samples described above.

Sand-Jensen (1998) found a distinctive longitudinal distribution pattern for mineral particles trapped within macrophyte stands. Therefore a sampling regime of longitudinal cores through the stand was developed. A total of five cores were taken on each sampling occasion. The long axis of the stand was divided into four equidistant sections and a core taken from each of these sections. The downstream trailing section of the *Ranunculus* became the fifth coring location.

As the sampling season progressed it was noted that organic rich deposits were accumulating at the stream margin. Preliminary examination of this material showed it to contain large numbers of blackfly faecal pellets. To ascertain the magnitude of these deposits a marginal sample was collected from each of the four sampling sites whenever these deposits were present. The sampling, storage and processing of these samples was identical to that of the *Ranunculus* cores.

All samples were returned to the laboratory and stored in their sample bottles until ready for analysis.

3.2.2 Laboratory analysis of samples

A volumetric sub-sampling technique was used to measure abundance and volume of faecal pellets. The first stage in processing the suspended samples in the laboratory was to carefully decant excess IMS from the sample bottles whilst ensuring that none of the collected material was lost. The samples were then agitated to resuspend the particles from the base of the bottle and the resulting suspension poured into a funnel placed in a Simport 17 x 100 mm polypropylene sterile culture tube. The sample bottle was rinsed with IMS and the remaining material washed into the tube. After the particles in suspension had settled to the bottom of the tube the IMS was adjusted until exactly10 ml of liquid was contained with the tube. The culture tubes were inverted 5 times to ensure homogenous mixing of the sample and then a 1 ml sub-sample was removed using a Gilson p1000 pipette and transferred to a Sedgewick Rafter Counting Cell. Sedgewick Rafter Cells are typically used for determining phytoplankton abundance (Antenucci et al., 2005), and therefore are suitable for counting particles in suspension.

Tests on the sub-sampling efficiency of this technique revealed the coefficient of variation to be 20.2%, an error value considered to be within acceptable boundaries for

the sub-sampling of this material due to the very high numbers of pellets contained within the samples.

An Olympus SZ40 dissecting microscope was used to process the samples. To determine the volume of faecal pellets the first 20 pellets encountered in the counting cell had their longest and shortest axes measured with an eyepiece micrometer. Blackfly faecal pellets typically have one axis longer then the other (Ladle & Griffiths, 1980) therefore the volume of the pellets was calculated assuming them to be cylindrical. All of the remaining pellets were counted using a tally counter. The total number of pellets in the cell was multiplied by 10 to give the total number of pellets in the water sample and this was divided by the number of litres to give faecal pellet abundance as pellets L^{-1}.

Core samples were washed out of the sample jars, using a hose attached to a tap, onto a 20 cm diameter brass sieve, with a mesh aperture of 1 mm. The sample was rinsed so the coarse material was retained on the sieve while the finer material was washed into an 8 L bucket. The elutriant within the bucket was poured through a 25 μm Monyl mesh net and rinsed under the hose to remove very fine particles. Material retained in the net was transferred into 450 ml wide-mouthed plastic bottles with screw lids.

The residual material contained large quantities of organic and mineral particles, necessitating the separation of the organic fraction from the mineral. Techniques for the separation of macroinvertebrates from samples were adapted for the separation of the largely organic material. These involve placing the sample into a liquid of high specific gravity and agitating the sample to suspend the macroinvertebrates, the resulting suspension can then be decanted with the denser mineral particles left in behind. These techniques have been developed for soil macroinvertebrates which have a specific

gravity of less than 1.1, therefore liquids with a specific gravity of around 1.2, such as saturated sucrose solutions are used (Edwards, 1991). However, blackfly faecal pellets have a considerably greater specific gravity of 1.33 (Ladle et al., 1987), necessitating a solution with a higher specific gravity than sucrose. Zinc sulphate solutions (specific gravity 1.4) are used in veterinary science to separate parasite eggs from soil samples (Nunes et al., 1994). It has the additional advantages of being cheap to buy and non-toxic and so was selected for the separating liquid in this study.

The plastic containers containing the core sample and zinc sulphate solution were inverted five times to ensure complete mixing of the sample. The lid unscrewed and the elutriant, predominantly organic matter, was slowly decanted into the Monyl net. The excess zinc sulphate was allowed to drain away and the process repeated until only a mineral dominated deposit was left in the plastic containers. The elutriant was transferred to 250 ml glass screw top bottles and the volume of zinc sulphate made up to 150 ml. This material was sub-sampled using the same technique employed for the sampling of material from the water column (see above).

During trials it was noted that some faecal pellets were still present within the mineral deposits left after the sample was separated, necessitating the sampling of this material. The mineral deposits were washed into the Monyl net with tap water, allowed to drain and transferred into a 9 cm diameter glass petri dish and covered. The petri dish was picked up and shaken vertically for ten seconds. The dish was placed back on the bench, the cover removed and a 20 cm diameter plastic ring was randomly placed into the dish from which a sub-sample was removed. The coefficient of variation for this sub-sampling technique was 16.4%. Material contained within the ring was transferred using

a disposable plastic pipette to a 6 cm plastic petri dish marked with a grid pattern. From this dish the volume of 20 pellets was measured and the total number of pellets counted.

To determine what proportion of the suspended load in these systems was in the form of faecal pellets it was necessary to determine the mass of the faecal pellets. A 25 L sample of chalk stream water was collected from the Bere Stream along with larvae attached to the trailing stems of *Ranunculus*. These were transferred to the laboratory and the *Ranunculus* leaves with attached larvae, were placed in Perspex tanks filled with stream water. Vigorous water movement was maintained by circulating water using aquarium pumps and air stones.

To produce pellets, c.50 larvae were transferred to a 3L Pyrex conical flask. The flask was filled with the Bere Stream water and an air stone inserted to produce a current. The gut throughput time of blackfly larvae is stated as being between 20 mins to 1 hr (Ladle et al., 1972; Wotton, 1978), therefore larvae were left for approximately one and a half hours to allow them to feed on the new food source and to complete the egestion of previously ingested material. At the end of this period the conical flask was vigorously swirled and all water and particles poured away. The flask was then refilled with the same stream water ensuring that pellets produced consist entirely of the material from the Bere Stream as all material ingested prior to the experiment will have been excreted and replaced with material from the newly added water. The flask was left overnight to allow the larvae to produce enough pellets to allow determination of their mass.

Pellets were harvested by swirling the conical flask to resuspend any particles on the bottom of the flask. As the quantities of material to be weighed were very small it was necessary to use the larger faecal pellets to obtain the most accurate results. The contents of the flask were therefore poured through a 106 μm sieve to retain the larger

pellets. Using a wash bottle containing distilled water, the pellets were transferred into a glass petri dish and placed under a dissecting microscope.

Calculations on the quantity of material needed to determine mass were based on the work by Ladle et al. (1987). These data showed that five large pellets would provide enough material to be accurately recorded by the balance. Measurements were made on a Sartorius SC2 Microbalance, this gives a reading down to 0.1 μg and so was able to accurately record the mass of the faecal pellets (>10 μg).

The volumes of five faecal pellets were recorded using a calibrated eyepiece micrometer and then gently manoeuvred together with a dissecting needle. The pellets were drawn into a Pasteur pipette until the water level in the pipette reached a pre-marked graduation on the pipette edge, this ensured that the same volume of water was drawn into the pipette on each occasion. The pellets were then transferred to a pre-weighed, pre-dried Thermo Finnigan Universal tin container. For each faecal pellet sample collected a sample of water from the petri dish was taken up into the pipette to the graduation and placed into to another pre-weighed, pre-dried tin container. This allowed for errors in mass caused by the addition of water to the containers to be controlled. A total of five replicates were used for both the faecal pellets and the blank controls. All samples were placed in to an oven at 105 °C and dried overnight (Cotton pers. comm.). Samples were allowed to cool and were then reweighed, the mass of the empty containers and blank controls were subtracted to provide a mass value for the known volume of pellets.

The storage of the core material in IMS prevented the proportion of the organic load as faecal pellets to be determined by mass calculations as there will have been leaching of organic material into the IMS. Instead a volumetric determination of the proportions

was used. Perspex tubes of either 14.5 or 26 mm diameter were sealed at the bottom with a rubber bung and a 2 cm layer of sand poured in. The sand ensured that there would be a uniform base on which the core samples could settle. Core samples were tipped into the tubes and the tubes were filled to the top with tap water. The tubes were then inverted repeatedly until the material was homogeneously mixed. The tubes were then stood vertically using a retort stand and spirit level and left overnight to settle. The settling produced a distinctive grading of the samples with the heavier organic particles at the base of the core and the lighter organic particles on top. The depth of the organic layer was measured three times using measuring callipers and the mean depth used to determine the quantity of organic matter in the core. The values of faecal pellet abundance and volume for the cores was used to determine total volume of faecal pellets from which the proportion as faecal pellets could be calculated.

3.3 Results

3.3.1 Abundance of faecal pellet in suspension

The abundance of pellets in suspension at Bere Stream showed a clear seasonal pattern (Figure 3.1). Analysis of the data with one-way ANOVA revealed highly significant differences between the abundance of faecal pellets found at different months of the year ($P = < 0.001$) (Table 3.1). Faecal pellet numbers rose gradually over the course of the year to a relatively steady plateau of around 2000 pellets L^{-1} from May to August. Numbers started to rise again in September and continued to rise, reaching a maximum in November when there were over 6039 pellets L^{-1} until declining through until February (Figure 3.1).

To reveal significant differences between the monthly samples the data were analysed using *post-hoc* Fisher Least Significant Difference test (Fisher LSD). This showed that there were no significant differences ($P = > 0.05$) between the abundance of faecal pellets in September, October, November and December. There were also no significant differences ($P = > 0.05$) between abundance in June, July and August and between May and July (Table 3.2). The influence of macrophyte cover and discharge on the abundance of faecal pellets in transport was seen by overlaying these data on to the abundance histogram (Figure 3.1). A ratio of the annual maximum value to the monthly value was used to examine the impact of changes in the parameters on abundance. These show that the abundance of faecal pellets closely mirrored the rise of the macrophyte cover and was negatively associated with the discharge. Midway through the period of peak faecal pellet abundance there is the start of a steep decline in macrophyte cover and a concurrent increase in discharge (Figure 3.1).

Figure 3.1; Annual variation in faecal pellet abundance in suspension, Closed circles indicate the ratio of maximum annual *Ranunculus* cover to monthly *Ranunculus* cover and open circles show the ratio of maximum discharge to monthly discharge. Bere Stream, March 2003 – February 2004. Error bars = 95% confidence intervals.

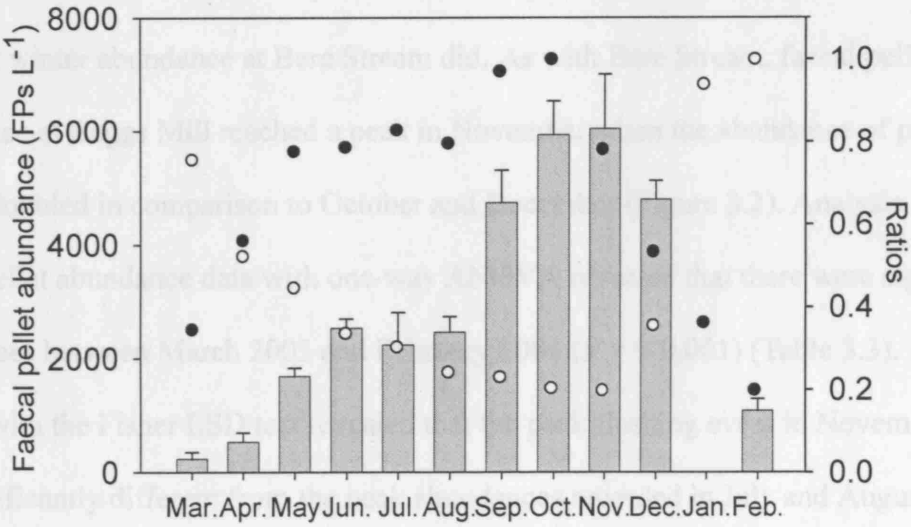


Table 3.1; Results of one-way ANOVA comparing the abundance of faecal pellets in suspension between months at Bere Stream.

Source	D.F.	SS	MS	F	P
Month	10	44.528	4.453	64.920	< 0.001
Error	43	2.949	0.069		
Total	53	47.478			

Table 3.2; Results of *post-hoc* Fisher LSD test showing significant differences in the abundance of faecal pellets between months at Bere Stream; L = Faecal pellet abundance in the column months are significantly lower than the abundance in the row months; G = Faecal pellet abundance in the column months are significantly greater than those in the row months; Blank spaces indicate that there was no significant differences between the samples (P = 0.05).

Apr.										
May	L	L								
Jun.	L	L	L							
Jul.	L	L								
Aug.	L	L	L							
Sep.	L	L	L	L	L	L				
Oct.	L	L	L	L	L	L				
Nov.	L	L	L	L	L	L				
Dec.	L	L	L	L	L	L				
Feb.	L	L	G	G	G	G	G	G	G	G
	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.

At Baggs Mill (Figure 3.2) peak faecal pellet abundance did not occur during the winter period but was reached in July and August with 3128 & 2826 pellets L^{-1} respectively. There was a period of high faecal pellet abundance over the autumn / winter period, although it did not dominate the pattern of faecal pellet abundance to the same extent as the peak winter abundance at Bere Stream did. As with Bere Stream, faecal pellet abundance at Baggs Mill reached a peak in November when the abundance of pellets almost doubled in comparison to October and December (Figure 3.2). Analysis of the faecal pellet abundance data with one-way ANOVA revealed that there were significant differences between March 2003 and February 2004 ($P = < 0.001$) (Table 3.3). *Post-hoc* testing with the Fisher LSD test revealed that the peak flushing event in November was not significantly different from the peak abundances recorded in July and August (Table 3.4). Due to difficulties sampling within the channel there are only limited macrophyte and discharge data available. Both reach a peak in July, and macrophyte cover declines steadily over the remaining sampling period. Discharge declines in August and then remains approximately steady until November when it rises substantially. This coincides with the increase in faecal pellet abundance in November (Figure 3.2).

Figure 3.2; Annual variation in faecal pellet abundance, Closed circles indicate the ratio of maximum annual *Ranunculus* cover to monthly *Ranunculus* cover and open circles show the ratio of maximum discharge to monthly discharge. Baggs Mill, March 2003 – February 2004. Error bars = 95% confidence intervals.

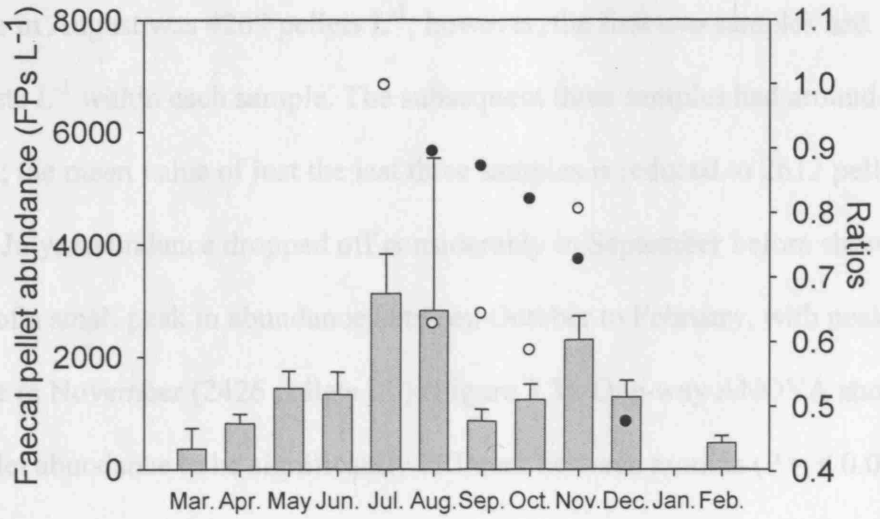


Table 3.3; Results of one-way ANOVA comparing the abundance of faecal pellets in suspension between months at Baggs Mill.

Source	D.F.	SS	*MS*	F	P
Month	10	18.493	1.849	26.700	< 0.001
Error	41	2.840	0.069		
Total	51	21.333			

Table 3.4; Results of *post-hoc* Fisher LSD test showing significant differences in the abundance of faecal pellets between months at Baggs Mill; L = Faecal pellet abundance in the column months are significantly lower than the abundance in the row months; G = Faecal pellet abundance in the column months are significantly greater than those in the row months; Blank spaces indicate that there was no significant differences between the samples (P = 0.05).

Apr.	L									
May	L	L								
Jun.	L	L								
Jul.	L	L	L	L						
Aug.	L	L	L	L						
Sep.	L		G	G	G	G				
Oct.	L	L			G	G	L			
Nov.	L	L	L	L			L	L		
Dec.	L	L			G	G	L		G	
Feb.		G	G	G	G	G	G	G	G	G
	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.

During the early part of the year, March to July, at Maiden Newton there was substantial inter-monthly variation in faecal pellet abundance (Figure 3.3). There was a peak in August, although the samples showed substantial variation. Mean faecal pellet abundance in August was 4269 pellets L^{-1}; however, the first two samples had 7000 – 8000 pellets L^{-1} within each sample. The subsequent three samples had around 2000 pellets L^{-1}; the mean value of just the last three samples is reduced to 2612 pellets L^{-1}, similar to July. Abundance dropped off considerably in September before showing evidence of a small peak in abundance between October to February, with peak abundance in November (2426 pellets L^{-1}) (Figure 3.3). One-way ANOVA showed faecal pellet abundance to be significantly different between months ($P = < 0.001$) (Table 3.5), although the data did not show any clear patterns at Maiden Newton, as confirmed by the results of the Fisher LSD test (Table 3.6). The macrophyte cover shows an initial drop between July and August this is accompanied by an increase in faecal pellet abundance in August. The next significant decrease is between October and November which is again accompanied by an increase in abundance (Figure 3.3).

Figure 3.3; Annual variation in faecal pellet abundance, Closed circles indicate the ratio of maximum annual *Ranunculus* cover to monthly *Ranunculus* cover and open circles show the ratio of maximum discharge to monthly discharge. Maiden Newton, March 2003 – February 2004. Error bars = 95% confidence intervals.

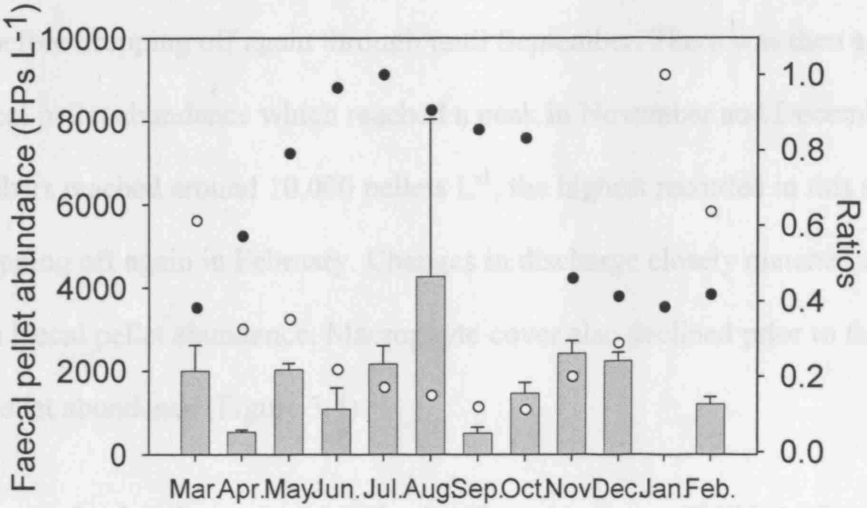


Table 3.5; Results of one-way ANOVA comparing the abundance of faecal pellets in suspension between months at Maiden Newton.

Source	D.F.	SS	MS	F	P
Month	10	19.345	1.935	24.040	< 0.001
Error	44	3.540	0.081		
Total	54	22.885			

Table 3.6; Results of *post-hoc* Fisher LSD test showing significant differences in the abundance of faecal pellets between months at Maiden Newton; L = Faecal pellet abundance in the column months are significantly lower than the abundance in the row months; G = Faecal pellet abundance in the column months are significantly greater than those in the row months; Blank spaces indicate that there was no significant differences between the samples (P = 0.05).

Apr.	G									
May		L								
Jun.	G	L	G							
Jul.		L		L						
Aug.	L	L	L	L	L					
Sep.	G		G	G	G	G				
Oct.		L			G	G	L			
Nov.		L		L			L	L		
Dec.		L		L		G	L	L		
Feb.	G	L	G		G	G	L		G	G
	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.

Faecal pellet abundance at East Stoke (Figure 3.4) demonstrated a clear seasonal pattern. Low numbers were present at the beginning of the year in March (501 pellets L^{-1}) and April (611 pellets L^{-1}) these then rose rapidly to over 3000 pellets L^{-1} for May and June before dropping off again through until September. There was then a dramatic rise in faecal pellet abundance which reached a peak in November and December when pellet numbers reached around 10,000 pellets L^{-1}, the highest recorded in this study, before dropping off again in February. Changes in discharge closely matched the changes in faecal pellet abundance. Macrophyte cover also declined prior to the increase in faecal pellet abundance (Figure 3.4).

The differences in abundance were highly significant, one-way ANOVA ($P = < 0.001$) (Table 3.7). The Fisher LSD test showed that the summer peak in May and June had significantly higher faecal pellet abundance then the rest of the year with the exception of the peak in November and December which was higher then all of the other sampling occasions (Table 3.8).

Figure 3.4; Annual variation in mean faecal pellet abundance, Closed circles indicate the ratio of maximum annual *Ranunculus* cover to monthly *Ranunculus* cover and open circles show the ratio of maximum discharge to monthly discharge. East Stoke, March 2003 – February 2004. Error bars = 95% confidence intervals.

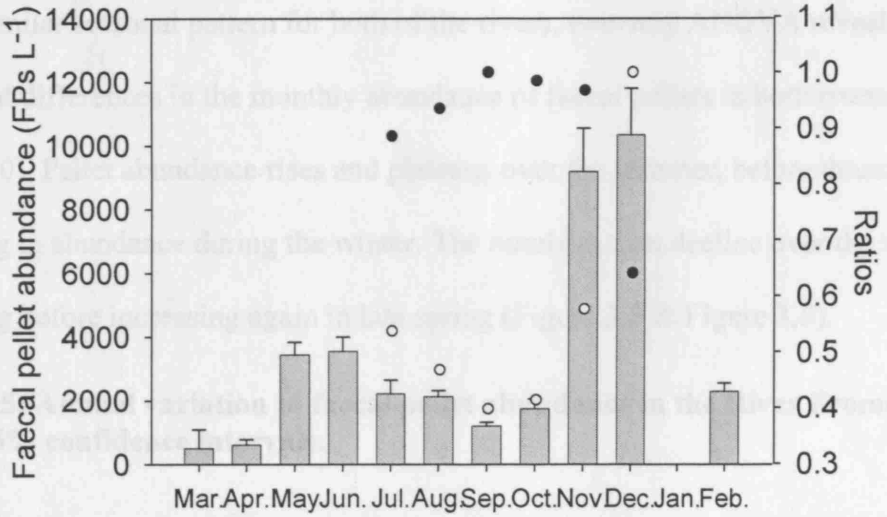


Table 3.7; Results of one-way ANOVA comparing the abundance of faecal pellets in suspension between months at East Stoke.

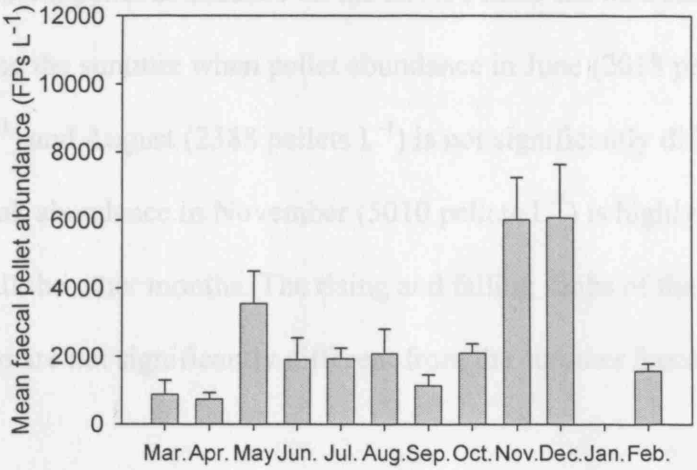
Source	D.F.	SS	MS	F	P
Month	10	41.615	4.162	165.560	< 0.001
Error	42	1.056	0.025		
Total	52	42.671			

Table 3.8; Results of *post-hoc* Fisher LSD test showing significant differences in the abundance of faecal pellets between months at East Stoke; L = Faecal pellet abundance in the column months are significantly lower than the abundance in the row months; G = Faecal pellet abundance in the column months are significantly greater than those in the row months; Blank spaces indicate that there was no significant differences between the samples (P = 0.05).

	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Apr.	L									
May	L	L								
Jun.	L	L								
Jul.	L	L	G	G						
Aug.	L	L	G	G						
Sep.	L	L	G	G	G	G				
Oct.	L	L	G	G	G	G	L			
Nov.	L	L	L	L	L	L	L	L		
Dec.	L	L	L	L	L	L	L	L		
Feb.	L	L	G	G			L	L	G	G

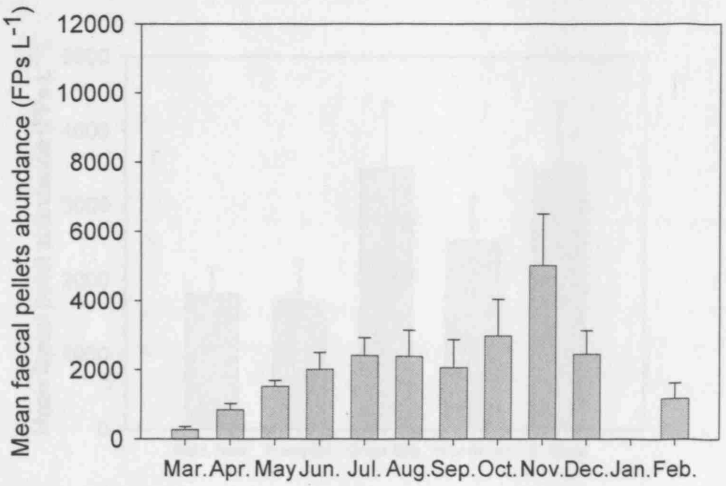
To determine annual variation in faecal pellet abundance at the catchment scale the abundance data for the two main sites and those for the intermediate sites were combined for both the River Frome (Figure 3.5) and Piddle (Figure 3.6). These data show a similar seasonal pattern for both of the rivers, two-way ANOVA revealed significant differences in the monthly abundance of faecal pellets in both rivers (Table 3.9 & 3.10). Pellet abundance rises and plateaus over the summer, before dramatically increasing in abundance during the winter. The numbers then decline over the winter and spring before increasing again in late spring (Figure 3.5 & Figure 3.6).

Figure 3.5; Annual variation in faecal pellet abundance in the River Frome, Error bars = 95% confidence intervals.



On the River Frome July (1929 pellets L^{-1}) and August (2077 pellets L^{-1}) had no significant difference in faecal pellet abundance and although there was a significant difference between these months and June (1895 pellets L^{-1}) this was not highly significant. November (6029 pellets L^{-1}) and December (6085 pellets L^{-1}) were not significantly different but were highly significantly different from all the other months. July and August were not significantly different from October (2092 pellets L^{-1}) and February (1573 pellets L^{-1}), which coincided with the rising and falling limb of the winter peak abundance (Table 3.11A).

Figure 3.6; Annual variation in faecal pellet abundance in the River Piddle, March 2003 – February 2004. Error bars = 95% confidence intervals.



The pattern of faecal pellet abundance on the River Piddle shows a similar trend. There is a period during the summer when pellet abundance in June (2018 pellets L^{-1}), July (2413 pellets L^{-1}) and August (2388 pellets L^{-1}) is not significantly different between the months. The peak abundance in November (5010 pellets L^{-1}) is highly significantly different from all the other months. The rising and falling limbs of the winter peak abundance again are not significantly different from the summer faecal pellet abundance (Table 3.12A).

Faecal pellet abundance across the catchments showed no clear differences or trends in faecal pellet numbers travelling from upstream to downstream. On the River Frome (Figure 3.7) the sites with the highest abundance are situated at the lower end of the catchment while on the Piddle they are at the top end of the catchment (Figure 3.8). There were significant differences between the sites (Table 3.9 & 3.10), however the Tukey post-hoc test confirmed the random pattern of abundance seen in the histogram (Table 3.11B & 3.12B).

Figure 3.7; Downstream annual variation in faecal pellet abundance in suspension in the River Frome, March 2003 – February 2004. Error bars = 95% confidence intervals.

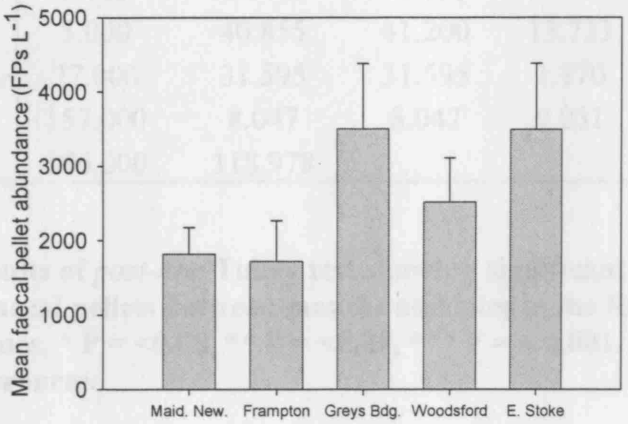


Figure 3.8; Downstream annual variation in faecal pellet abundance in suspension in the River Piddle, March 2003 – February 2004. Error bars = 95% confidence intervals.

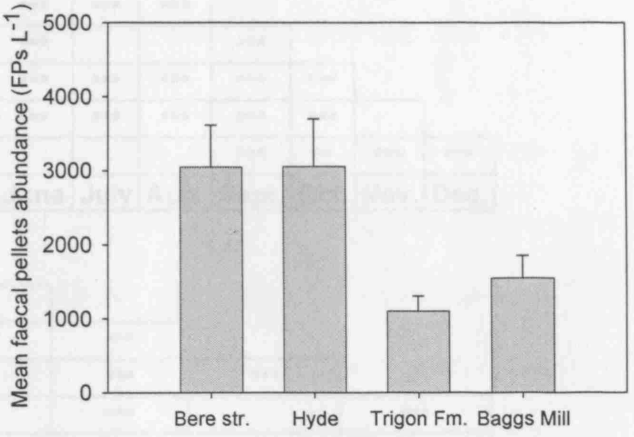


Table 3.9; Results of two-way ANOVA comparing the abundance of faecal pellets in suspension between months and between sites in the River Frome.

Source	D.F.	Seq SS	Adj SS	Adj MS	F	P
Month	10.000	114.861	120.149	12.015	224.470	< 0.001
Location	4.000	25.424	24.269	6.067	113.350	< 0.001
Month*Location	40.000	68.958	68.958	1.724	32.210	< 0.001
Error	210.000	11.241	11.241	0.054		
Total	264.000	220.485				

Table 3.10; Results of two-way ANOVA comparing the abundance of faecal pellets in suspension between months and between sites in the River Piddle.

Source	D.F.	Seq SS	Adj SS	Adj MS	F	P
Month	9.000	38.481	40.638	4.515	88.100	< 0.001
Location	3.000	40.855	41.200	13.733	267.960	< 0.001
Month*Location	27.000	31.595	31.595	1.170	22.830	< 0.001
Error	157.000	8.047	8.047	0.051		
Total	196.000	118.978				

Table 3.11; Results of *post-hoc* Tukey test showing significant differences in the abundance of faecal pellets between months and sites in the River Frome. Asterisks represent P values, * P = <0.05, ** P = <0.01, *** P = < 0.001. Blank spaces = no significant differences.

A

April										
May	***	***								
June	**	***	***							
July	***	***	***	**						
Aug.	***	***	***	**						
Sept.	***	***	***	***	***	***				
Oct.	***	***	***	***			***			
Nov.	***	***	***	***	***	***	***	***		
Dec.	***	***	***	***	***	***	***	***		
Feb.	***	***	***				***	**	***	***
	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.

B

Frampton	***			
Greys Bdg.	***	***		
Woodsford	**	***	***	
East Stoke	***	***		***
	Maiden Newton	Frampton	Greys Bdg.	Woodsford

Table 3.12; Results of *post-hoc* Tukey test showing significant differences in the abundance of faecal pellets between months and sites in the River Piddle. Asterisks represent P values, * P = <0.05, ** P = <0.01, *** P = < 0.001. Blank spaces = no significant differences.

A

May	***								
June	***								
July	***	***							
Aug.	***	*							
Sept.	***			***	*				
Oct.	***	***				**			
Nov.	***	***	***	***	***	***	***		
Dec.	***	***				***		***	
Feb.		***	***	***	***	***	***	***	***
	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.

B

Hyde			
Trigon Farm	***	***	
Baggs Mill	***	***	***
	Bere str.	Hyde	Trigon Farm

3.3.1.1 Abundance of faecal pellets upstream and downstream of Ranunculus stands

There was no clear pattern in the abundance of faecal pellets found upstream and downstream of a *Ranunculus* stand at Bere Stream (Figure 3.9). As the data were not normally distributed Wilcoxon signed ranks test was used to test for differences. This showed no significant differences between samples collected upstream and samples collected downstream of the *Ranunculus* stands (P = 0.574). There were no clear patterns in the abundance of faecal pellets found upstream and downstream of the *Ranunculus* stand at Baggs Mill. Some months had higher abundance upstream of the stand and others downstream of the stand (Figure 3.10).

Figure 3.9; Faecal pellet abundance upstream and downstream of a *Ranunculus* stand at Bere Stream, Error bars = 95% confidence intervals. The downstream values correspond with those used in Figure 3.1.

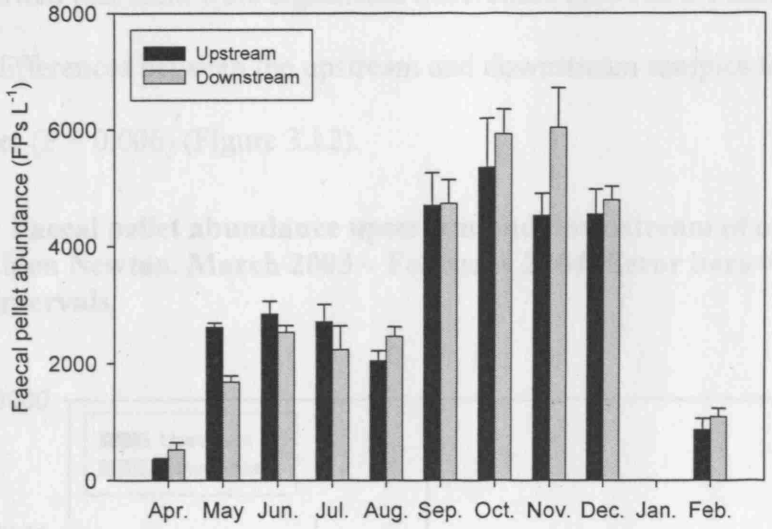
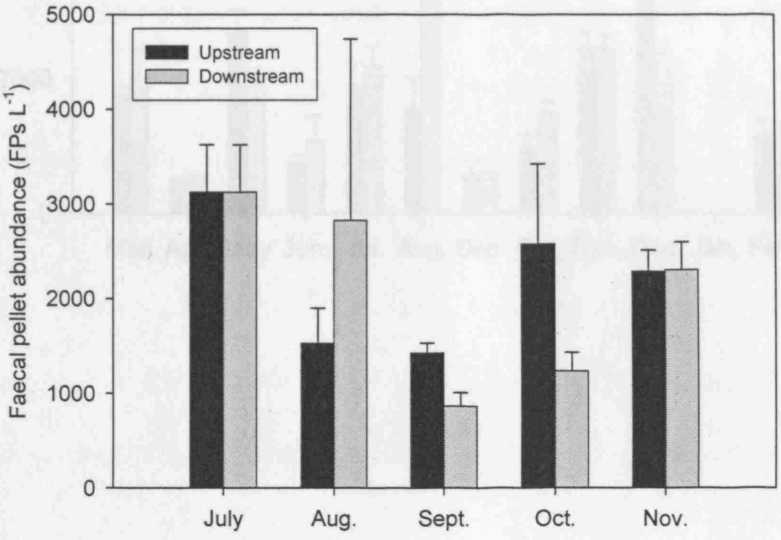


Figure 3.10; Faecal pellet abundance upstream and downstream of a Ranunculus stand at Baggs Mill. March 2003 – November 2003, Error bars = 95% confidence intervals.



The data were tested using the Wilcoxon signed ranks test, this showed that these differences were not significant (P = 0.053). In keeping with the other sites Wilcoxon signed ranks test showed there to be no significant differences between samples collected upstream and samples collected downstream of the *Ranunculus* stand at

Maiden Newton ($P = 0.18$) (Figure 3.11). At East Stoke analysis of the data on faecal pellet abundance upstream and downstream of the *Ranunculus* using a Wilcoxon signed ranks test showed that there were significant differences between the samples as a result of the large differences between the upstream and downstream samples for November and December ($P = 0.006$) (Figure 3.12).

Figure 3.11; Faecal pellet abundance upstream and downstream of a Ranunculus stand at Maiden Newton. March 2003 – February 2004, Error bars = 95% confidence intervals.

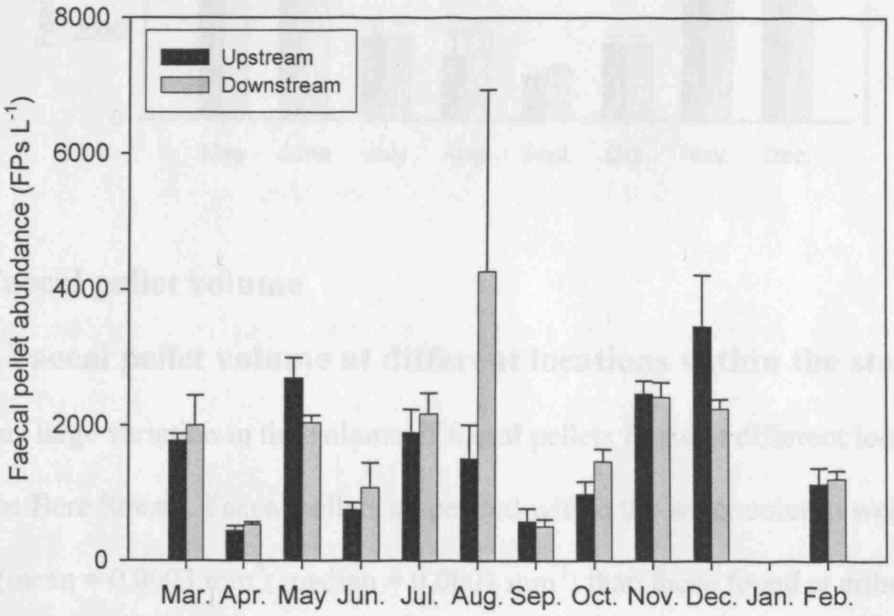
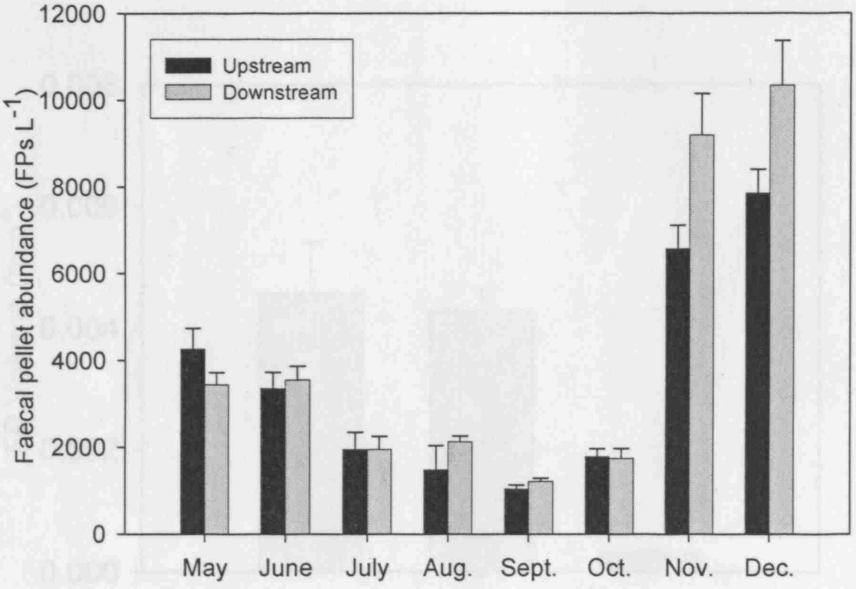


Figure 3.12; Faecal pellet abundance upstream and downstream of a Ranunculus stand at East Stoke. Error bars = 95% confidence intervals.

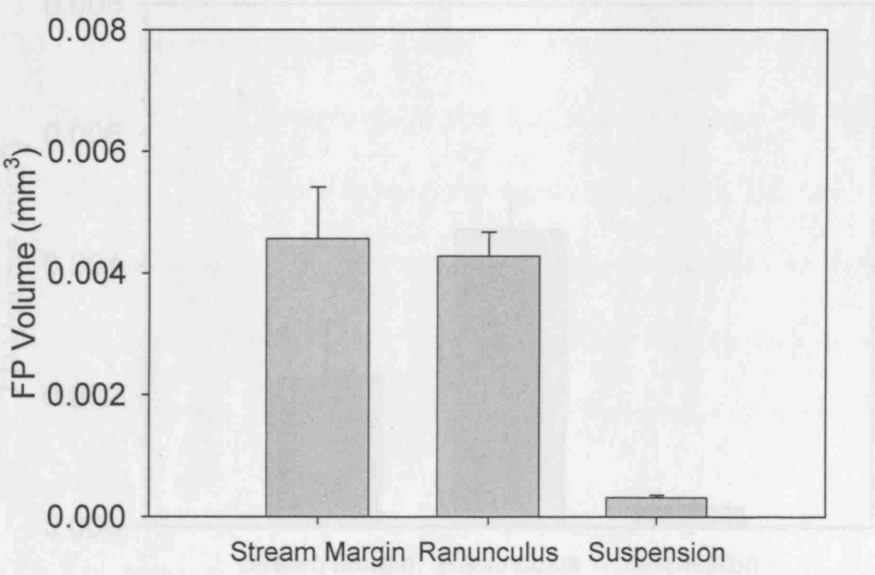


3.3.2 Faecal pellet volume

3.3.2.1 Faecal pellet volume at different locations within the stand

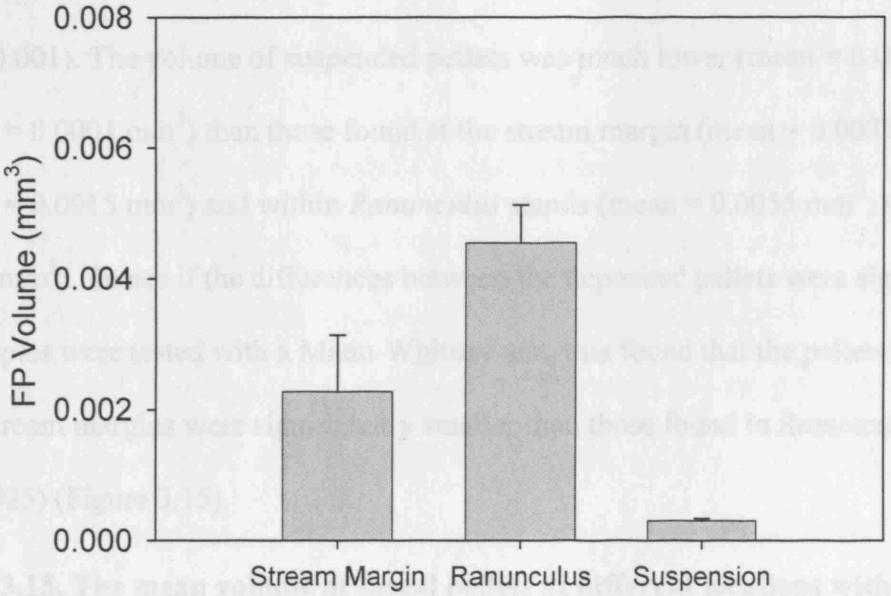
There was large variation in the volume of faecal pellets found at different locations within the Bere Stream. Faecal pellets suspended within the water column were much smaller (mean = 0.0003 mm^3; median = 0.0001 mm^3) than those found at either the channel margin (mean = 0.0046 mm^3; median = 0.0025 mm^3) or trapped within stands of *Ranunculus* (mean = 0.0043 mm^3; median = 0.0016 mm^3). In turn pellets deposited at the stream margin were larger in volume than those trapped within *Ranunculus* stands (Figure 3.13).

Figure 3.13; The mean volume of individual faecal pellets at different locations within Bere Stream, March 2003 – February 2004. Error bars = 95% confidence intervals.



The volumes of the faecal pellets were not normally distributed and did not conform to the requirements for an ANOVA test. Therefore the data were tested using a Kruskal-Wallis test. This revealed highly significant differences between the volumes of faecal pellets found at different locations within Bere Stream ($P = < 0.001$). There are clear differences between the size of suspended pellets and those found deposited at the stream margin and within *Ranunculus* stands. To test for significant differences between the volumes of pellets deposited within *Ranunculus* stands and those at the stream margin the samples were tested using a Mann-Whitney test. This found highly significant differences between the volumes of the pellets at the two locations ($P = < 0.001$).

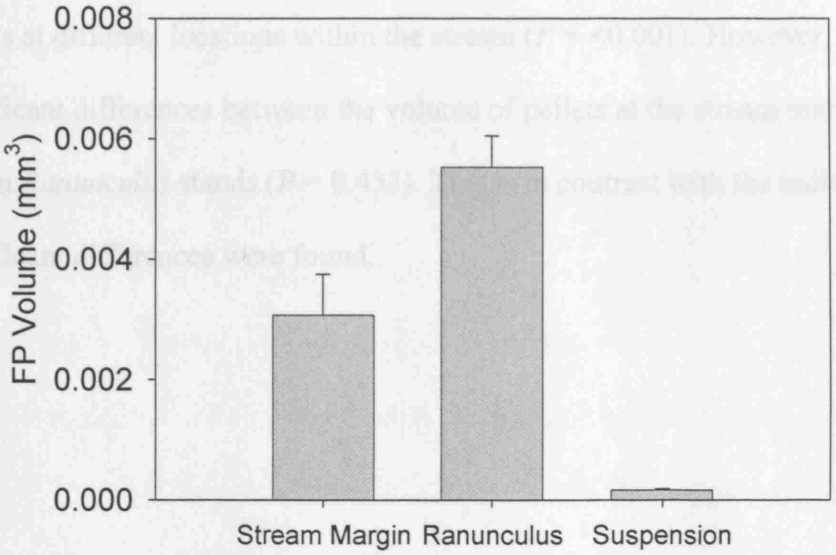
Figure 3.14, The mean volume of individual faecal pellets at different locations within Baggs Mill from March 2003 – February 2004. Error bars = 95% confidence intervals.



As with Bere Stream the volume of faecal pellets at Baggs Mill showed variation in volumes dependent on their location within the stream. Faecal pellets suspended within the water column were smaller (mean = 0.0003 mm^3; median = 0.0001 mm^3) then those found at the channel margin (mean = 0.0023 mm^3; median = 0.0007 mm^3) or trapped within stands of *Ranunculus* (mean = 0.0045 mm^3; median = 0.0023 mm^3). Pellets trapped within *Ranunculus* stands were larger in volume than those deposited at the stream margin (Figure 3.14). The volumes of the faecal pellets were tested using a Kruskal-Wallis test. This revealed highly significant differences between the volumes of faecal pellets found at different locations within Baggs Mill ($P = < 0.001$). There are clear differences between the size of suspended pellets and those found deposited at the stream margin and within *Ranunculus* stands. The differences between the deposited pellets were tested using a Mann-Whitney test which found highly significant differences between the volumes of pellets found at the two locations ($P = < 0.001$).

The volumes of the faecal pellets at Maiden Newton were tested using a Kruskal-Wallis test for nonparametric data. This revealed highly significant differences between the individual volume of faecal pellets found at three different locations at Maiden Newton (P = < 0.001). The volume of suspended pellets was much lower (mean = 0.0002 mm^3; median = 0.0001 mm^3) than those found at the stream margin (mean = 0.0031 mm^3; median = 0.0015 mm^3) and within *Ranunculus* stands (mean = 0.0055 mm^3; median = 0.0018 mm^3). To see if the differences between the deposited pellets were significant the samples were tested with a Mann-Whitney test, this found that the pellets deposited at the stream margins were significantly smaller than those found in *Ranunculus* stands (P = 0.025) (Figure 3.15).

Figure 3.15, The mean volume of faecal pellets at different locations within Maiden Newton, March 2003 – February 2004. Error bars = 95% confidence intervals.

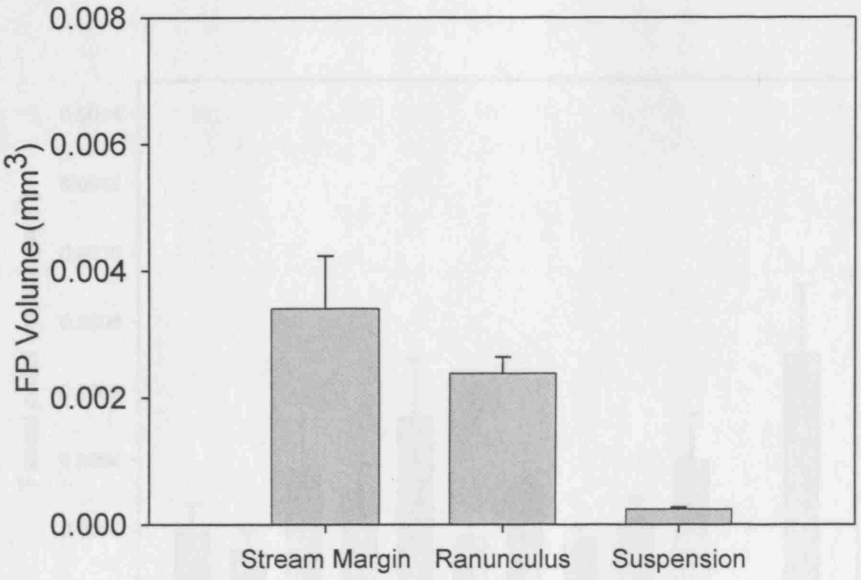


Analysis of the data using a Kruskal-Wallis test revealed highly significant differences between the volumes of faecal pellets found at different locations within East Stoke (*P* = < 0.001). The suspended pellets had a smaller volume (mean = 0.0002 mm^3; median = 0.0001 mm^3) than those found deposited at the stream margin (mean = 0.0034 mm^3;

median = 0.0014 mm^3) and within *Ranunculus* stands (mean = 0.0024 mm^3; median = 0.0011 mm^3) (Figure 3.16). The *Ranunculus* and marginal pellets were tested using a Mann-Whitney test which did not find significant differences between the volumes of pellets found at the two locations ($P = 0.193$).

The pellet volumes found within each of the four main sampling sites on the Frome and Piddle were combined to provide an overview of the size differences of pellets found within discrete locations within the channel. The smallest pellets, by an order of magnitude, were those in suspension (mean = 0.0003 mm^3; median = 0.0001 mm^3), these were followed by those pellets found at the stream margin (mean = 0.0035 mm^3; median = 0.0016 mm^3), while the largest pellets were trapped in *Ranunculus* stands (mean = 0.0044 mm^3; median = 0.0016 mm^3). Analysis of the data using the Kruskal-Wallis test revealed that there were significant differences between the volume of pellets at different locations within the stream ($P = <0.001$). However, there were no significant differences between the volume of pellets at the stream margin and those within *Ranunculus* stands ($P = 0.453$). This is in contrast with the individual sites where significant differences were found.

Figure 3.16, The mean volume of faecal pellets at different locations within East Stoke, March 2003 – February 2004. Error bars = 95% confidence intervals.



3.3.2.2 Volume of faecal pellets in suspension

The volume of faecal pellets in suspension at Bere Stream showed substantial variation over the year although there was no clear annual pattern. The lowest value was found in April (Mean volume = 0.00014 mm^3), after which monthly mean volumes varied over the year until the highest mean volume was reached in February (Mean volume = 0.00071 mm^3) (Figure 3.17). Kruskal-Wallis test revealed highly significant annual variation in the volume of faecal pellets in suspension at Bere Stream ($P = < 0.001$). To test for pairwise differences in faecal pellet volume between months the data were tested using Mann-Whitney tests (Table 3.13). This showed a complex pattern of significant differences between the months although February was highly significantly different from all of the other months. There were no data available for January faecal pellet volumes.

Figure 3.17; Annual variation in the mean volume of faecal pellets in suspension at Bere Stream, March 2003 – February 2004, Error bars = 95% confidence intervals.

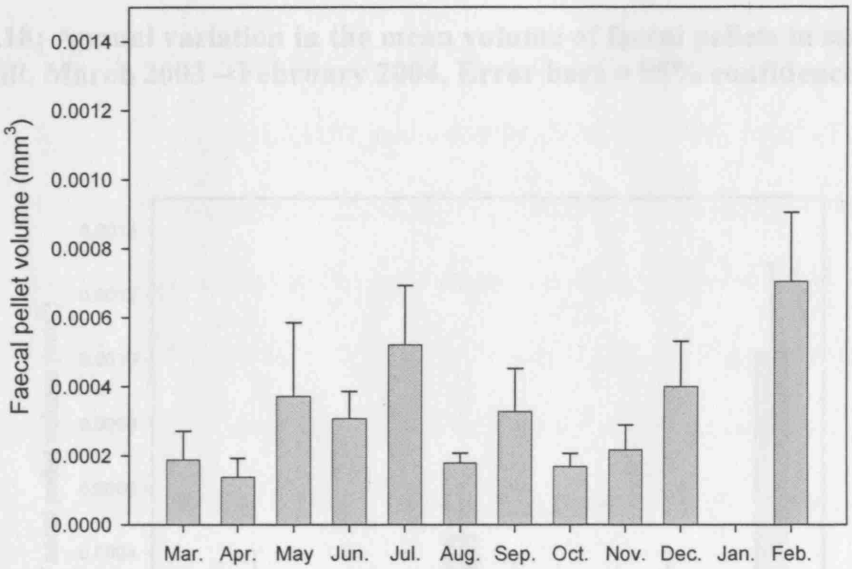


Table 3.13; Results of *post-hoc* Mann Whitney tests showing significant differences in the volume of faecal pellets in suspension between months at Bere Stream. Asterisks represent P values, * P = <0.05, ** P = <0.01, *** P = <0.001. Blank spaces are not significantly different.

Apr.										
May	**	***								
Jun.	***	***								
Jul.	***	***	*							
Aug.	***	***			*					
Sep.	***	***								
Oct.		***		***	***	*	**			
Nov.	*	***		*	**					
Dec.	***	***	*			**		***	***	
Feb.	***	***	***	***	***	***	***	***	***	***
	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.

The pattern of faecal pellet volume in suspension from March to December was varied at Baggs Mill. Faecal pellet volume varied from 0.0001 mm^3 in March to 0.0004 mm^3 in August, February had by far the largest pellets in suspension with a mean volume of 0.001 mm^3 (Figure 3.18). Kruskal-Wallis test showed these differences to be highly significant (P = < 0.001). To test for pairwise differences in faecal pellet volume between months the data were tested using Mann-Whitney tests (Table 3.14). These

revealed a complex pattern of significant differences between the months, however as with Bere Stream February was the only month where the pellets were all highly significantly different in volume from all the other months.

Figure 3.18; Annual variation in the mean volume of faecal pellets in suspension at Baggs Mill. March 2003 – February 2004, Error bars = 95% confidence intervals.

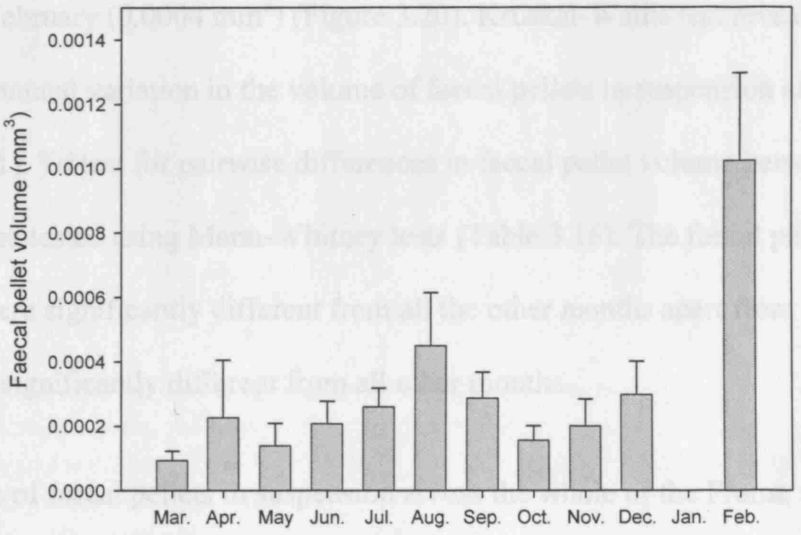


Table 3.14; Results of *post-hoc* Mann-Whitney tests showing A) significant differences in the volume of faecal pellets in suspension between months at Baggs Mill. Asterisks represent P values, * P = <0.05, ** P = <0.01, *** P = < 0.001. Blank spaces are not significantly different.

Apr.										
May										
Jun.	***	*	**							
Jul.	***	***	***	**						
Aug.	***	***	***	***						
Sep.	***	***	***	*		*				
Oct.				*	***	***	***			
Nov.	***	*	*		**	***	*	*		
Dec.	***	***	***	**				***	**	
Feb.	***	***	***	***	***	***	***	***	***	***
	Mar.	**Apr.**	**May**	**Jun.**	**Jul.**	**Aug.**	**Sep.**	**Oct.**	**Nov.**	**Dec.**

At Maiden Newton mean faecal pellet volume was 0.0001 mm^3 for every month except for July, October and December (0.0002 mm^3) and February 0.0003 mm^3 (Figure 3.19). The Kruskal-Wallis test revealed highly significant annual variation in the volume of faecal pellets in suspension at Maiden Newton ($P = < 0.001$). Differences in faecal

pellet volume between months were tested using Mann-Whitney tests, these showed July and February to be significantly different from all of the other months but not with each other (Table 3.15).

At East Stoke the mean volume of faecal pellets in suspension remained at between 0.0001 and 0.002mm^3 throughout the year apart from May (0.0003 mm^3), July (0.0005 mm^3) and February (0.0004 mm^3) (Figure 3.20). Kruskal-Wallis test revealed highly significant annual variation in the volume of faecal pellets in suspension at East Stoke ($P = < 0.001$). To test for pairwise differences in faecal pellet volume between months the data were tested using Mann-Whitney tests (Table 3.16). The faecal pellets in February were significantly different from all the other months apart from July, which in turn was significantly different from all other months.

The volume of faecal pellets in suspension across the whole of the Frome / Piddle catchment showed little variation. Over the spring and summer the faecal pellets had a mean volume of 0.0002 mm^3, except for July when this rose to 0.0004 mm^3. In December the volume rose to 0.0003 mm^3 and then rose again in February to a seasonal maximum of 0.0006 mm^3 (Figure 3.21). Kruskal-Wallis test revealed highly significant annual variation in the volume of faecal pellets in suspension in the Frome / Piddle catchment ($P = < 0.001$). To test for pairwise differences in faecal pellet volume between months the data were tested using Mann-Whitney tests (Table 3.17). These showed that the faecal pellets in suspension in July and February were significantly larger than the other faecal pellets sampled.

Figure 3.19; Annual variation in the mean volume of faecal pellets in suspension at Maiden Newton. March 2003 – February 2004, Error bars = 95% confidence intervals.

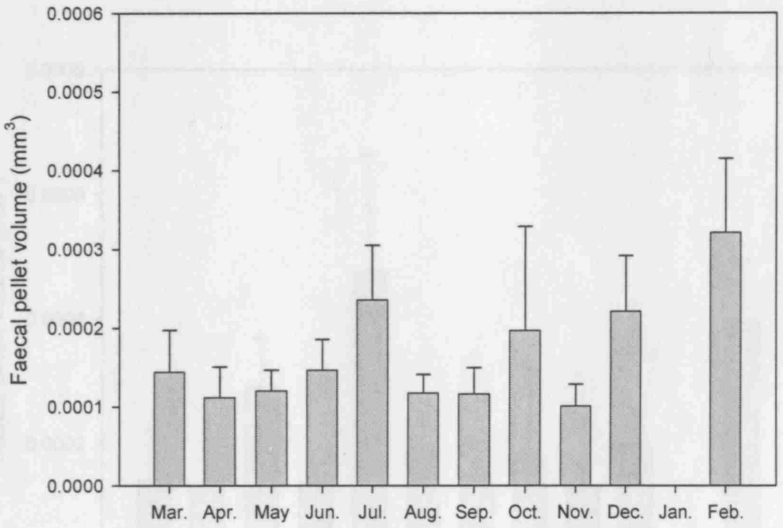


Table 3.15; Results of *post-hoc* Mann Whitney tests showing significant differences in the volume of faecal pellets in suspension between months at Maiden Newton. Asterisks represent P values, * P = <0.05, ** P = <0.01, *** P = < 0.001, blank space = no significant difference.

Apr.										
May										
Jun.										
Jul.	***	***	***	***						
Aug.					***					
Sep.					***					
Oct.				*	***	*				
Nov.			*	**	***	**	*			
Dec.	*	***	*		*		**	**	***	
Feb.	***	***	***	***		***	***	***	***	***
	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.

Figure 3.20; Annual variation in the mean volume of faecal pellets in suspension at East Stoke. Error bars = 95% confidence intervals.

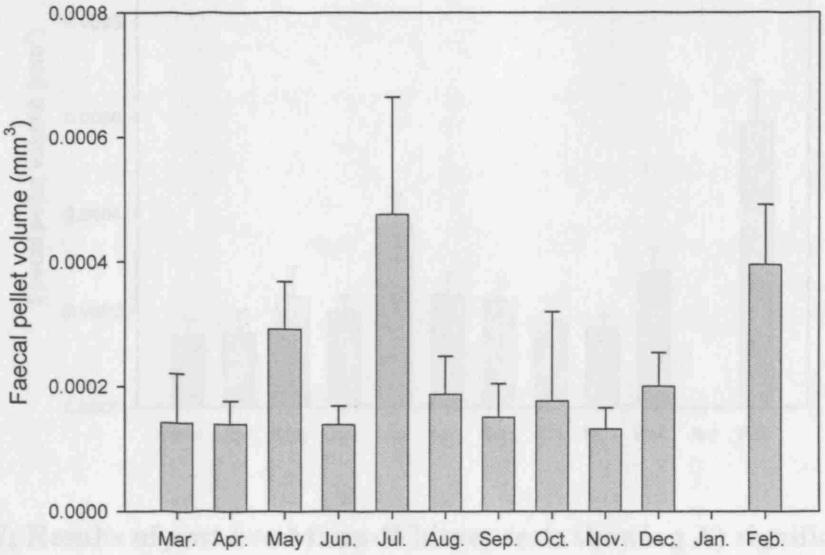


Table 3.16; Results of *post-hoc* Mann-Whitney tests showing A) significant differences in the volume of faecal pellets in suspension between months at East Stoke. Asterisks represent P values, * P = <0.05, ** P = <0.01, *** P = < 0.001. Blank spaces are not significantly different.

Apr.	***									
May	***	***								
Jun.	***	*	**							
Jul.	***	***	*	***						
Aug.	***	**	**		***					
Sep.	***		***		***					
Oct.	***		***	**	***	**	*			
Nov.	***		***		***	*				
Dec.	***	**	*		***			***	**	
Feb.	***	***	**	***		***	***	***	***	***
	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.

Figure 3.21; Annual variation in the mean volume of faecal pellets in suspension across the Frome / Piddle catchment. Error bars = 95% confidence intervals.

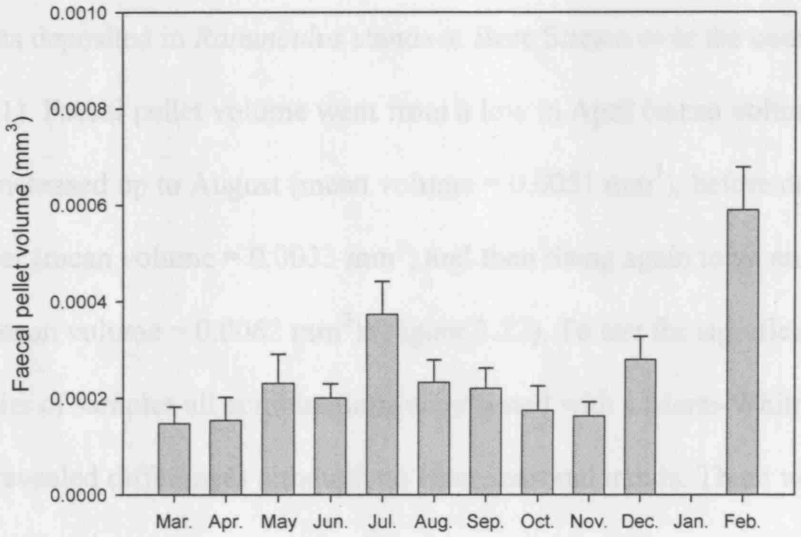


Table 3.17; Results of *post-hoc* Mann-Whitney tests showing A) significant differences in the volume of faecal pellets in suspension between months in the Frome / Piddle catchment. B) significant differences in the volume of faecal pellets in suspension in the Frome / Piddle catchment. Asterisks represent P values, * P = <0.05, ** P = <0.01, *** P = < 0.001. Blank space = no significant difference.

Apr.										
May	***	***								
Jun.	***	***								
Jul.	***	***	***	***						
Aug.	***	***	**		***					
Sep.	***	***			***					
Oct.	**		***	***	***	***	***			
Nov.	***	***	*	***	***	***	**			
Dec.	***	***	***	**	**		***	***	***	
Feb.	***	***	***	***	***	***	***	***	***	***
	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.

3.3.2.3 Variation in the volume of faecal pellets in *Ranunculus* stands

The Kruskal-Wallis test revealed highly significant differences between the volume of faecal pellets deposited in *Ranunculus* stands at Bere Stream over the course of a year (P = < 0.001). Faecal pellet volume went from a low in April (mean volume = 0.0022 mm^3) and increased up to August (mean volume = 0.0051 mm^3), before declining again in September (mean volume = 0.0033 mm^3) and then rising again to an annual high in February (mean volume = 0.0062 mm^3) (Figure 3.22). To test for significant differences between pairs of samples all combinations were tested with a Mann-Whitney test (Table 3.18), this revealed differences although no clear seasonal trends. There was no significant difference between faecal pellet volume and core location at Bere Stream (P = 0.21) (Figure 3.23).

Figure 3.22; Annual variation in the mean volume of faecal pellets trapped within *Ranunculus* stands at Bere Stream. March 2003 – February 2004, Error bars = 95% confidence intervals.

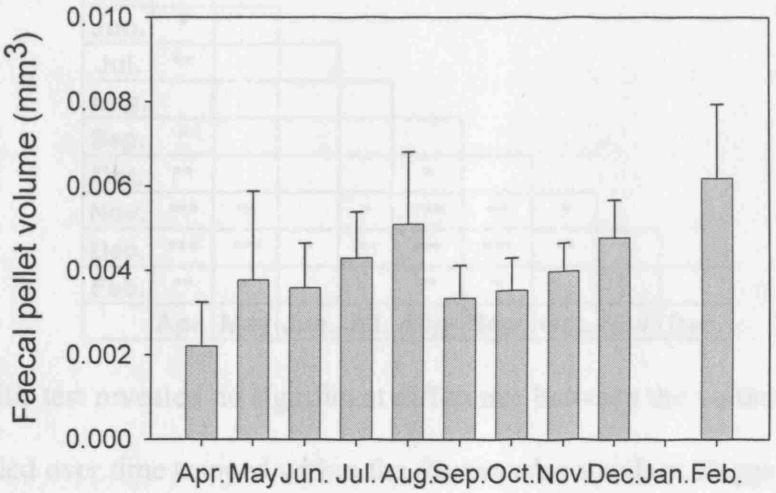


Figure 3.23; Annual longitudinal variation in mean faecal pellet volume within a *Ranunculus* stand, going from core 5 at the upstream end of the stand to core 1 at the downstream end of the stand. Bere Stream. Error bars = 95% confidence intervals.

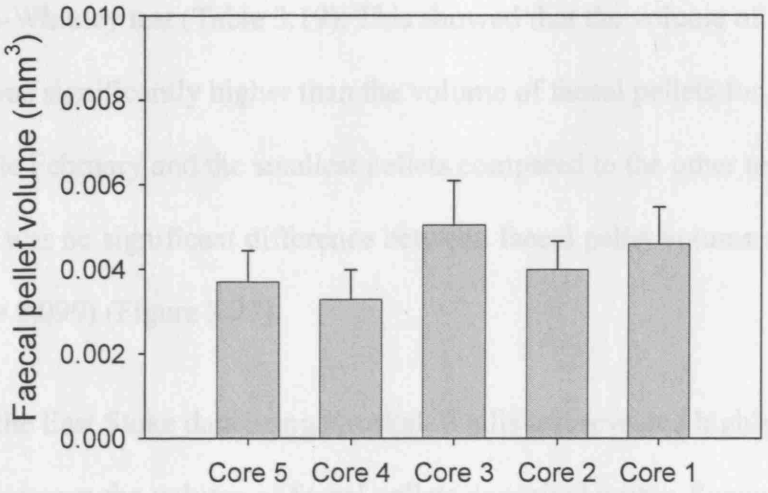


Table 3.18; Results of *post-hoc* Mann Whitney test showing significant differences in the volume of faecal pellets between months at Bere Stream. Asterisks represent P values, * P = <0.05, ** P = <0.01, *** P = < 0.001. Blank spaces are not significantly different.

	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
May									
Jun.	*								
Jul.	**								
Aug.									
Sep.	*								
Oct.	**				*				
Nov.	***	**		*	***	**	*		
Dec.	***	***	*	**	***	***	**		
Feb.	**	*			**	*			

Kruskal-Wallis test revealed no significant difference between the volume of faecal pellets sampled over time trapped within the *Ranunculus* stands at Baggs Mill (P = 0.804) These faecal pellets had a mean volume of 0.0045 mm^3 (Figure 3.24). There was no significant difference between faecal pellet volume and core location (P = 0.804) (Figure 3.25).

Kruskal-Wallis test revealed highly significant differences between the volume of faecal pellets over the course of a year within *Ranunculus* stands at Maiden Newton ($P = <$ 0.001). To test for differences between pairs of samples all combinations were tested with a Mann-Whitney test (Table 3.19). This showed that the volume of faecal pellets in September was significantly higher than the volume of faecal pellets for all the other months, while February and the smallest pellets compared to the other months (Figure 3.26). There was no significant difference between faecal pellet volume and core location ($P = 0.099$) (Figure 3.27).

Analysis of the East Stoke data using Kruskal-Wallis test revealed highly significant differences between the volume of faecal pellets deposited within *Ranunculus* stands over the course of a year (P = < 0.001) (Figure 3.28). To test for differences between pairs of samples all combinations were tested with a Mann-Whitney test. This showed that faecal pellets in August (0.003mm^3) were significantly larger than the faecal pellets found in the other months (All = 0.002 mm^3) (Table 3.20A). There was a highly significant difference between faecal pellet volume and core location ($P = < 0.001$) (Figure 3.29). To test for differences between pairs of samples all combinations were tested with a Mann-Whitney test (Table 3.20B). This showed that core 3 (0.003mm^3) was significantly larger than core 4 and core 5 (both = 0.002mm^3) which were immediately upstream of it but not from those downstream of it.

Across the Frome/Piddle catchment the Kruskal-Wallis test revealed highly significant differences between the volume of faecal pellets trapped in *Ranunculus* stands over the course of a year (P = < 0.001). To test for differences between pairs of samples all combinations were tested with a Mann-Whitney test (Table 3.21A). The overall pattern was of relatively little variation in the volume of faecal pellets, the clear exception was

for April which had pellets that were significantly smaller then all of the other months. There was a significant difference between faecal pellet volume and core location (P = 0.015). To test for differences between pairs of samples all combinations were tested with a Mann-Whitney test (Table 3.21B).

Figure 3.24; Annual variation in the mean volume of faecal pellets trapped within *Ranunculus* stands at Baggs Mill. March 2003 – November 2003 (all material had been washed out by November 2003). Error bars = 95% confidence intervals.

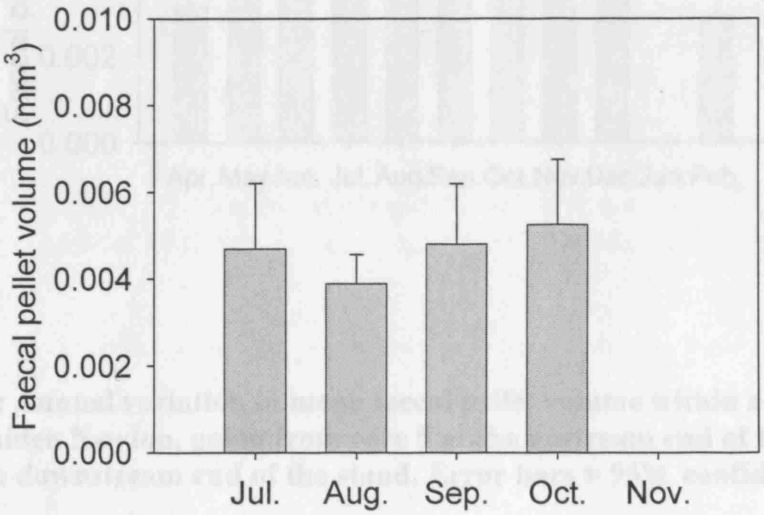


Figure 3.25; Annual longitudinal variation in mean faecal pellet volume within a *Ranunculus* stand, going from core 5 at the upstream end of the stand to core 1 at the downstream end of the stand between July 2003 – November 2004 at Baggs Mill (all material was washed out by November 2004). Error bars = 95% confidence intervals.

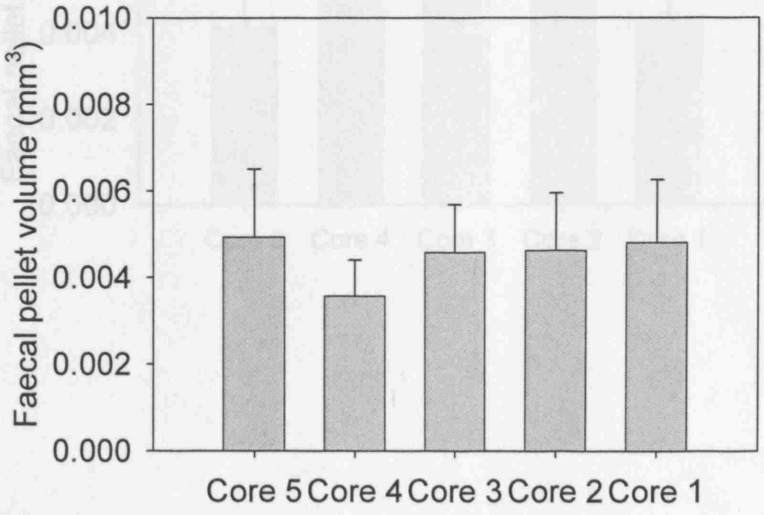


Figure 3.26; Annual variation in the mean volume of faecal pellets trapped within *Ranunculus* stands at Maiden Newton. March 2003 – February 2004, Error bars = 95% confidence intervals.

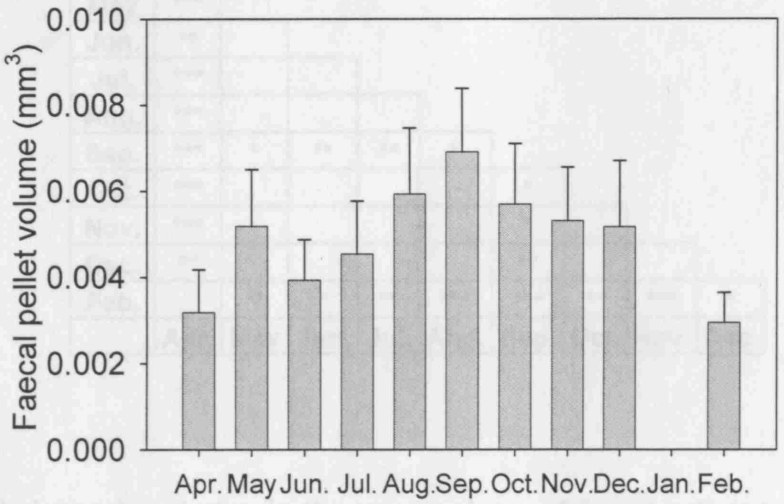


Figure 3.27; Annual variation in mean faecal pellet volume within a *Ranunculus* stand at Maiden Newton, going from core 5 at the upstream end of the stand to core 1 at the downstream end of the stand. Error bars = 95% confidence intervals.

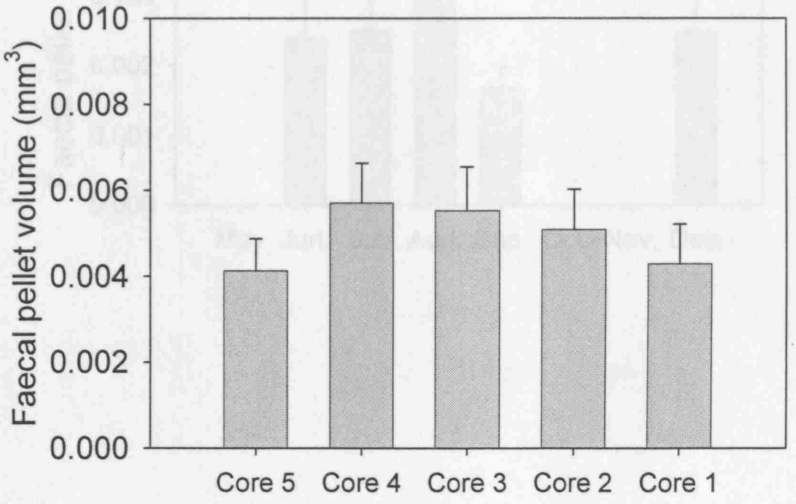


Table 3.19; Results of *post-hoc* Mann Whitney test showing significant differences in the volume of faecal pellets trapped within *Ranunculus* stands between months at Maiden Newton. Asterisks represent P values, * P = <0.05, ** P = <0.01, *** P = < 0.001.

May	***								
Jun.	**								
Jul.	***								
Aug.	***								
Sep.	***	*	**	**	*				
Oct.	***					*			
Nov.	***					*			
Dec.	**					**			
Feb.		**	*	**	***	***	**	***	*
	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.

Figure 3.28; Annual variation in the mean volume of faecal pellets trapped within *Ranunculus* stands at East Stoke. Error bars = 95% confidence intervals.

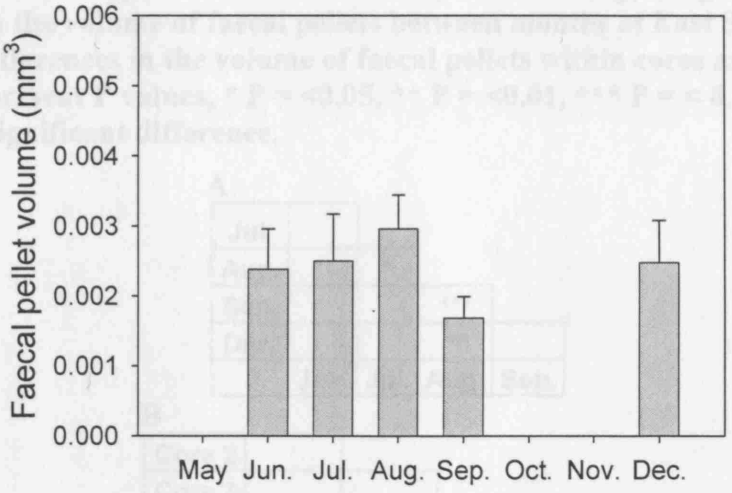


Figure 3.29; Longitudinal variation in mean faecal pellet volume within a *Ranunculus* stand, going from core 5 at the upstream end of the stand to core 1 at the downstream end of the stand. Error bars = 95% confidence intervals.

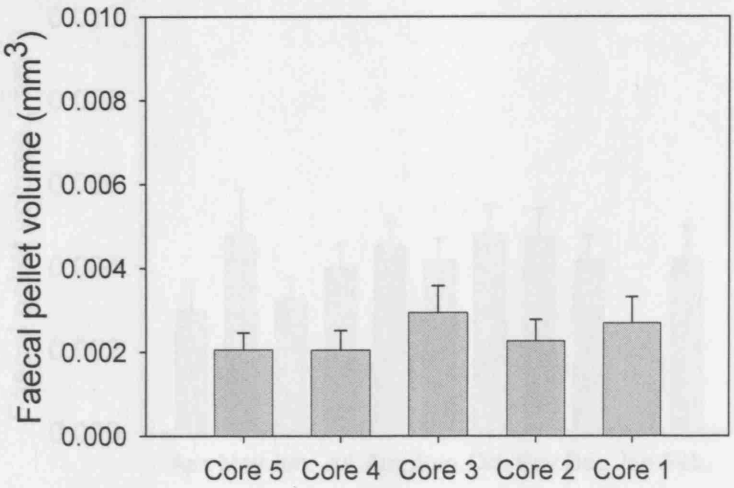


Table 3.20; Results of *post-hoc* Mann Whitney test showing A) significant differences in the volume of faecal pellets between months at East Stoke. B) significant differences in the volume of faecal pellets within cores at East Stoke Asterisks represent P values, * P = <0.05, ** P = <0.01, *** P = < 0.001. Blank spaces = no significant difference.

A

Jul.				
Aug.	**	*		
Sep.			***	
Dec.			**	
	Jun.	Jul.	Aug.	Sep.

B

Core 2				
Core 3				
Core 4			*	
Core 5			*	
	Core 1	Core 2	Core 3	Core 4

Figure 3.30; Annual variation in the mean volume of faecal pellets trapped within *Ranunculus* stands in the Frome / Piddle catchment. Error bars = 95% confidence intervals.

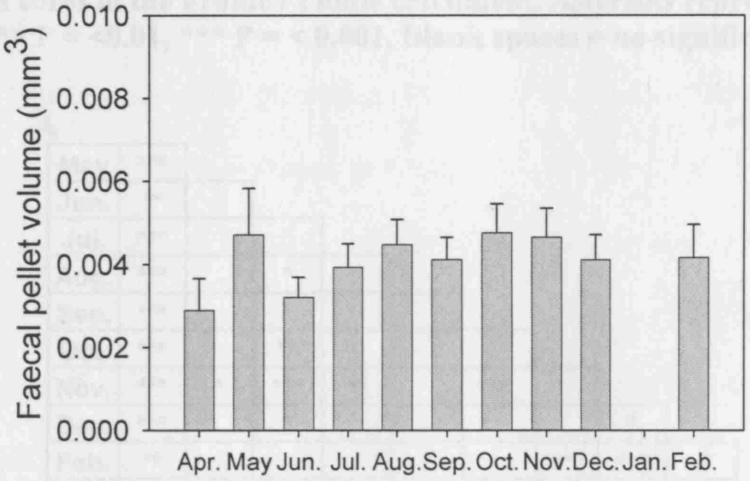


Figure 3.31; Longitudinal variation in mean faecal pellet volume within a *Ranunculus* stand, going from core 5 at the upstream end of the stand to core 1 at the downstream end of the stand. Error bars = 95% confidence intervals.

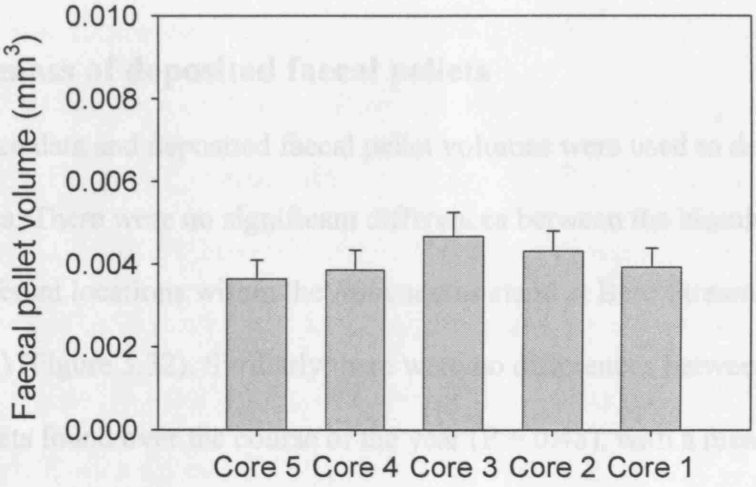


Table 3.21; Results of *post-hoc* Mann Whitney test showing A) significant differences in the volume of faecal pellets in *Ranunculus* stands between months in the Frome / Piddle catchment. B) significant differences in the volume of faecal pellets within cores in the Frome / Piddle catchment. Asterisks represent P values, * P = <0.05, ** P = <0.01, *** P = < 0.001. Blank spaces = no significant differences.

A

May	***							
Jun.	**							
Jul.	***							
Aug.	***		*					
Sep.	***							
Oct.	***		**					
Nov.	***	*	***	**		**		
Dec.	***		*					*
Feb.	**				*		*	***
	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.

B

core 2				
core 3				
core 4				
core 5	*		**	
	core 1	core 2	core 3	core 4

3.3.2.4 Biomass of deposited faecal pellets

The abundance data and deposited faecal pellet volumes were used to determine faecal pellet biomass. There were no significant differences between the biomass of faecal pellets at different locations within the *Ranunculus* stand at Bere Stream (P= 0.15) (Table 3.22A) (Figure 3.32). Similarly there were no differences between the biomass of faecal pellets found over the course of the year (P = 0.48), with a mean value of 28.8 g m^{-2} over the whole of the sampling period (Table 3.22B). The monthly data showed a great deal of variation with mean biomass within a stand varying from 3.0 g m^{-2} in February, to a high of 47.4 g m^{-2} in December. Individual values for a core reached as high as 190 g m^{-2} in June although mean values were much lower at 31.2 g m^{-2} (Figure 3.33). Bere Stream had much higher quantities of faecal pellets deposited at the stream

margins with a mean biomass of 230 g m^{-2}. The lowest value was 61.2 g m^{-2} and the highest 559 g m^{-2}, there were not enough samples to determine any patterns of annual variation.

At Baggs Mill the biomass of faecal pellets at different locations within the *Ranunculus* stand showed no significant differences, one-way ANOVA (P= 0.12) (Table 3.23A) (Figure 3.34). The biomass of faecal pellets found over the course of the year also showed no significant differences (P = 0.69) (Table 3.23B). The mean biomass value of 24.9 g m^{-2} was similar to that recorded at Bere Stream. By November the *Ranunculus* under study had lost so much biomass that no faecal pellets remained trapped within the stand. The monthly variation in the biomass trapped within stands varied from 17.4 g m^{-2} in October to 34.7 g m^{-2} in August. August also had the highest biomass value for a core of 73.5 g m^{-2} (Figure 3.35). There were only two cores taken of faecal pellets deposited at the stream margin, these gave a mean biomass of 378 g m^{-2}.

At Maiden Newton there were no significant differences between the biomass of faecal pellets at different locations within the *Ranunculus* stand (P= 0.36) (Table 3.24A) (Figure 3.36) or the monthly variation in the biomass of faecal pellets at Maiden Newton (P = 0.82) (Table 3.24B) (Figure 3.37). Lowest biomass values were found in April (3.6 g m^{-2}) with the highest in August (82.5 g m^{-2}), with a mean biomass of 28.8 g m^{-2}. Biomass values for faecal pellets deposited at the stream margins varied between 1.4 and 621 g m^{-2}, with a mean of 244 g m^{-2}.

At East Stoke there were no significant differences between the biomass of faecal pellets at different locations within the *Ranunculus* stand (P = 0.72) (Table 3.25A) (Figure 3.38) or the values found over the course of the year (P = 0.55) (Table 3.25B) (Figure 3.39). East Stoke had a substantially higher mean biomass of faecal pellets in

Ranunculus stands 140.2 g m^{-2} than all the other sites. These data on monthly variation showed annual variation with no material being present in the stand during May and then a build up of faecal pellets from June to August (210; 96 and 209 g m^{-2}). Values drop off in September (28 g m^{-2}) until no pellets are present in October and November although the biomass of pellets increases again in December (157 g m^{-2}) (Figure 3.34). There were high biomass values for the stream margins, with a mean of 200.8 g m^{-2}, a high of 324.6 g m^{-2} and a low of 100 g m^{-2}.

The Frome / Piddle data for biomass of faecal pellets were transformed using natural log to normalise the data and tested using a one-way ANOVA. This showed that there was no significant difference in biomass trapped within *Ranunculus* stands over the year (Table 3.26) (Figure 3.40) or between cores (Table 3.27) (Figure 3.41). *Ranunculus* stands have an average of 68.8 g m^{-2} dry weight of intact faecal pellets trapped within them over the course of a year. However, this value hides the considerable variation within stands, values varied from cores with no faecal pellets present to those with 890 g m^{-2} of intact faecal pellets.

Mean biomass values for pellets deposited at the stream margin are three-fold those found in *Ranunculus* with a mean value of 245.3 g m^{-2}. The highest and lowest values recorded were both at Maiden Newton when there was 621 g m^{-2} in October and 1.5 g m^{-2} in February. There were too few samples to be able to look at seasonal variation in the biomass at stream margins.

Figure 3.32; Annual mean faecal pellet biomass within a *Ranunculus* stand, going from core 5 at the upstream end of the stand to core 1 at the downstream end of the stand. Bere Stream. Error bars = 95% confidence intervals.

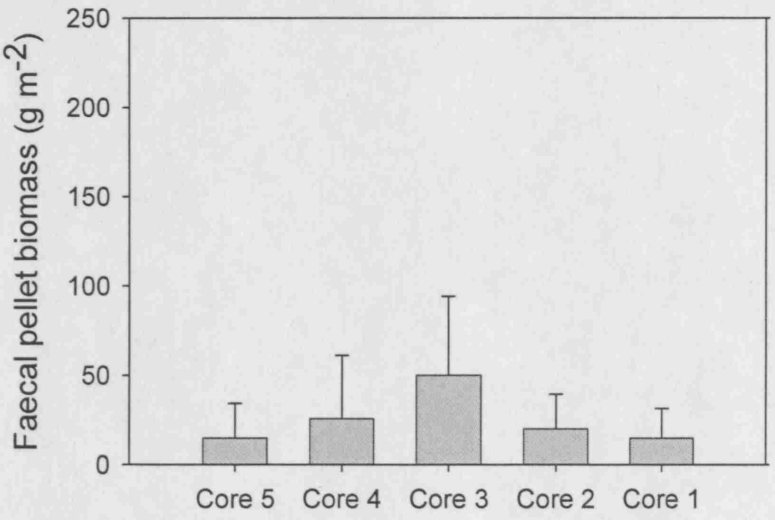


Figure 3.33; Mean annual faecal pellet biomass trapped within *Ranunculus* stands at Bere Stream. March 2003 – February 2004, Error bars = 95% confidence intervals.

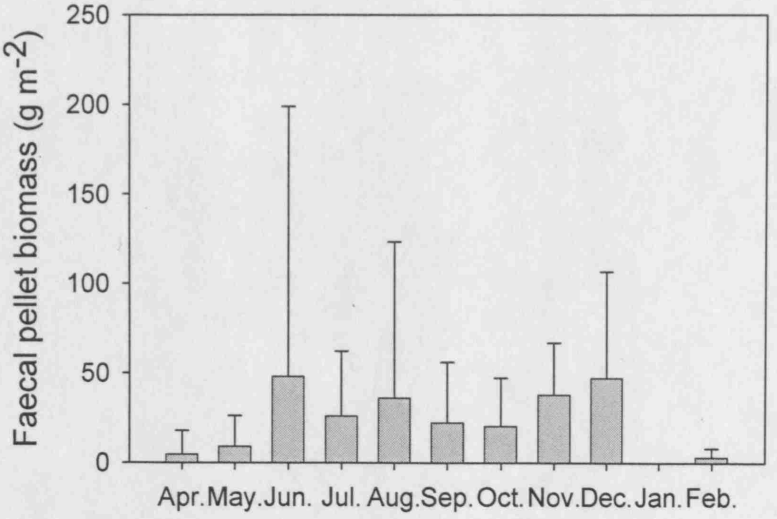


Table 3.22; Results of one-way ANOVA comparing A) the biomass of faecal pellets at different locations within *Ranunculus* stands at Bere Stream and B) the biomass of faecal pellets trapped within *Ranunculus* stands between months.

A

Source	D.F.	SS	MS	F	P
Core location	4.00	13.23	3.31	1.80	0.15
Error	34.00	62.50	1.84		
Total	38.00	75.72			

B

Source	D.F.	SS	MS	F	P
Month	9.00	17.52	1.95	0.97	0.48
Error	29.00	58.20	2.01		
Total	38.00	75.72			

Figure 3.34; Annual longitudinal variation in faecal pellet biomass within a *Ranunculus* stand, going from core 5 at the upstream end of the stand to core 1 at the downstream end of the stand, Baggs Mill. Error bars = 95% confidence intervals.

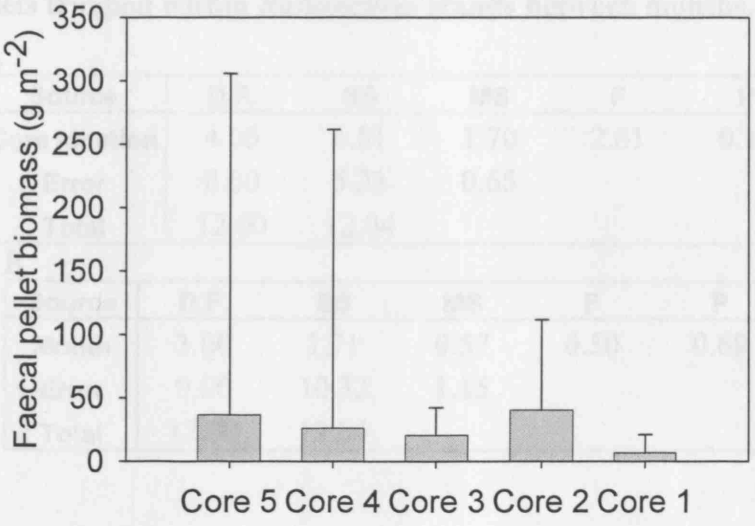


Figure 3.35; Mean monthly faecal pellet biomass trapped within *Ranunculus* stands at Baggs Mill. March 2003 – February 2004 (all material had been washed out by November) Error bars = 95% confidence intervals.

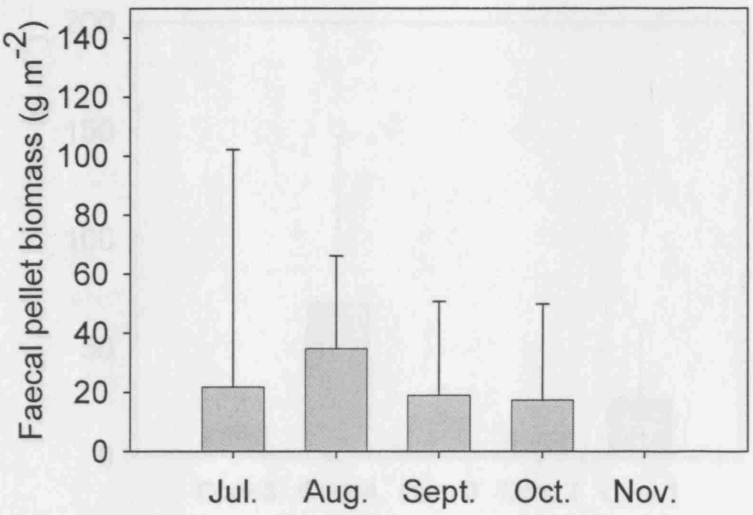


Table 3.23; Results of one-way ANOVA comparing A) the biomass of faecal pellets at different locations within *Ranunculus* stands at Baggs Mill and B) the biomass of faecal pellets trapped within *Ranunculus* stands between months.

A

Source	D.F.	SS	MS	F	P
Core location	4.00	6.81	1.70	2.61	0.12
Error	8.00	5.23	0.65		
Total	12.00	12.04			

B

Source	D.F.	SS	MS	F	P
Month	3.00	1.71	0.57	0.50	0.69
Error	9.00	10.32	1.15		
Total	12.00	12.04			

Figure 3.36; Longitudinal variation in faecal pellet biomass within a *Ranunculus* stand, going from core 5 at the upstream end of the stand to core 1 at the downstream end of the stand, Maiden Newton. Error bars = 95% confidence intervals.

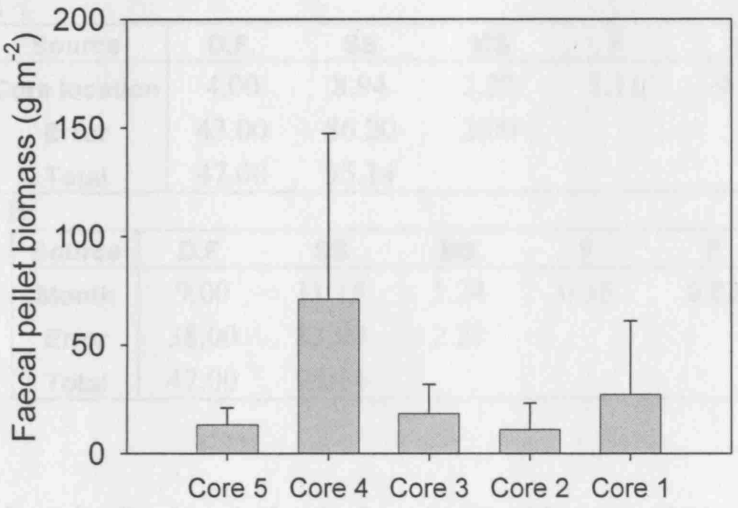


Figure 3.37; Total faecal pellet biomass trapped within *Ranunculus* stands at Maiden Newton. March 2003 – February 2004, Error bars = 95% confidence intervals.

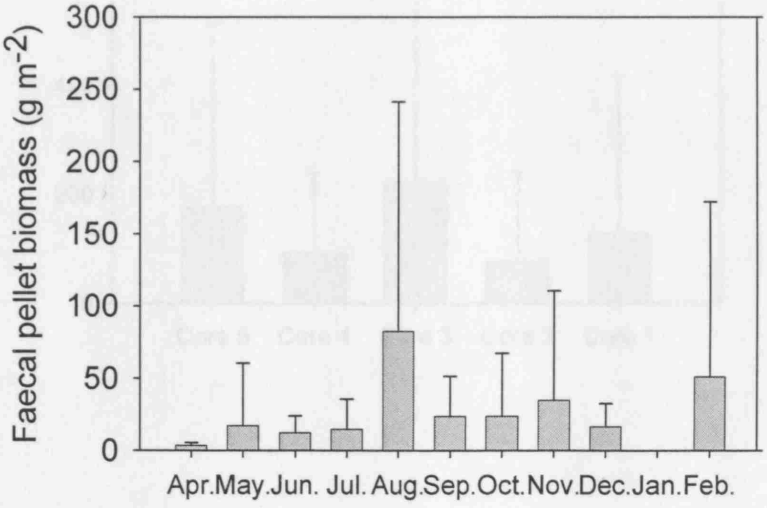


Table 3.24; Results of one-way ANOVA comparing A) the biomass of faecal pellets at different locations within *Ranunculus* stands and B) the biomass of faecal pellets trapped within *Ranunculus* stands between months at Maiden Newton.

A

Source	D.F.	SS	MS	F	P
Core location	4.00	8.94	2.23	1.11	0.36
Error	43.00	86.20	2.00		
Total	47.00	95.14			

B

Source	D.F.	SS	MS	F	P
Month	9.00	11.15	1.24	0.56	0.82
Error	38.00	83.99	2.21		
Total	47.00	95.14			

Figure 3.38; Longitudinal variation in faecal pellet biomass within a *Ranunculus* stand, going from core 5 at the upstream end of the stand to core 1 at the downstream end of the stand. Error bars = 95% confidence intervals.

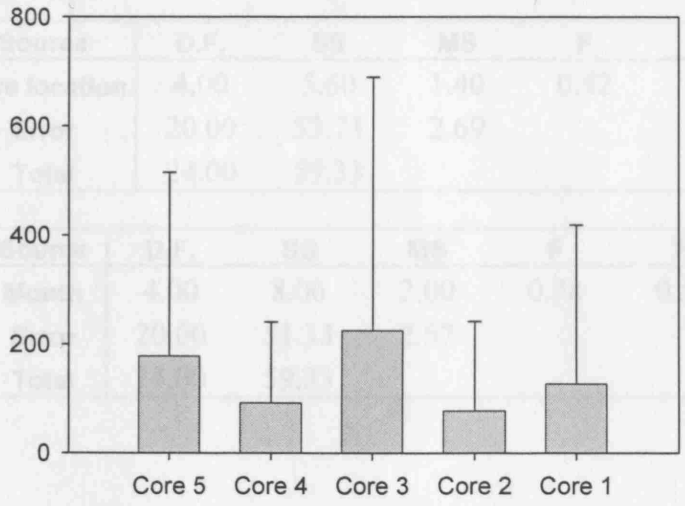


Figure 3.39; Total faecal pellet biomass trapped within *Ranunculus* stands at East Stoke, May – December 2003. Error bars = 95% confidence intervals.

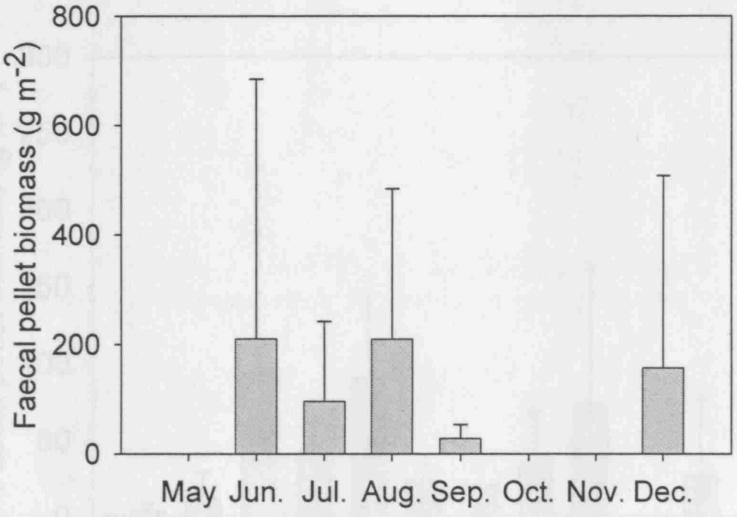


Table 3.25; Results of one-way ANOVA comparing A) the biomass of faecal pellets at different locations within *Ranunculus* stands and B) the biomass of faecal pellets trapped within *Ranunculus* stands between months at East Stoke.

A

Source	D.F.	SS	MS	F	P
Core location	4.00	5.60	1.40	0.52	0.72
Error	20.00	53.73	2.69		
Total	24.00	59.33			

B

Source	D.F.	SS	MS	F	P
Month	4.00	8.00	2.00	0.78	0.55
Error	20.00	51.33	2.57		
Total	24.00	59.33			

Figure 3.40; Total faecal pellet biomass trapped within *Ranunculus* stands across the Frome / Piddle catchment, April 2003 – February 2004. Error bars = 95% confidence intervals.

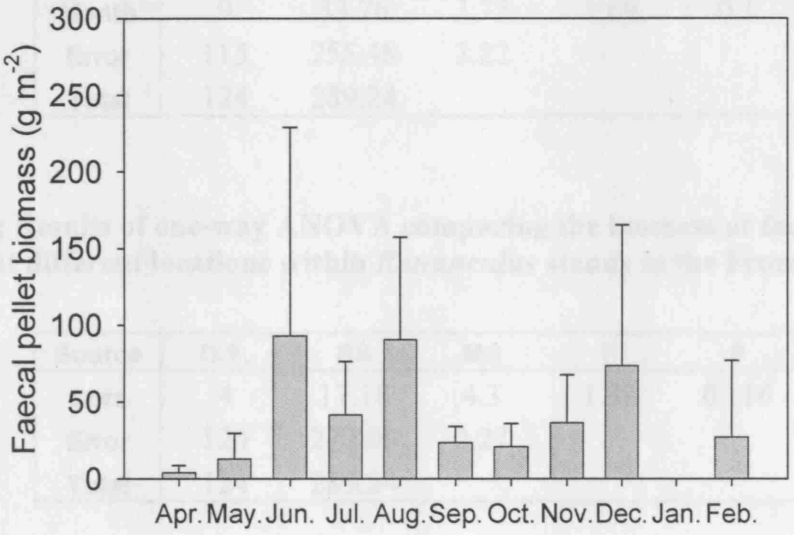


Figure 3.41; Longitudinal variation in faecal pellet biomass within a *Ranunculus* stand, going from core 5 at the upstream end of the stand to core 1 at the downstream end of the stand. Error bars = 95% confidence intervals.

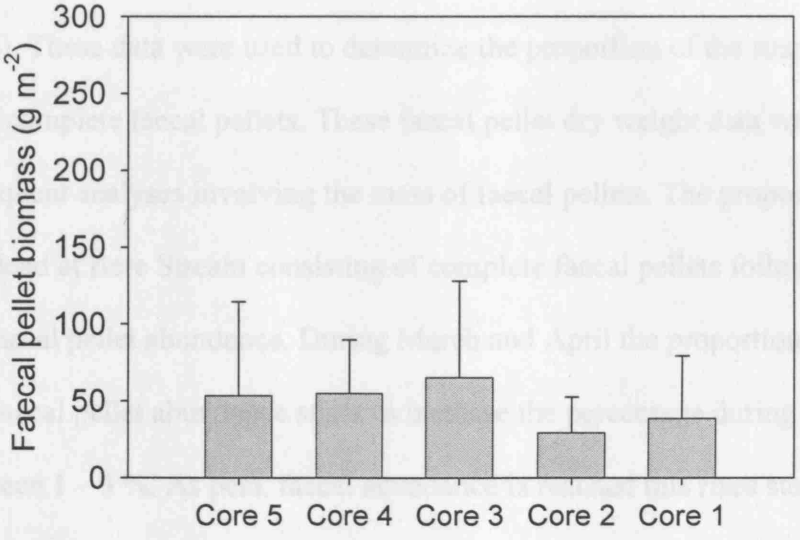


Table 3.26; Results of one-way ANOVA comparing the biomass of faecal pellets trapped within *Ranunculus* stands between months in the Frome / Piddle catchment.

Source	D.F.	SS	MS	F	P
Month	9	33.76	3.75	1.69	0.1
Error	115	255.48	2.22		
Total	124	289.24			

Table 3.27; Results of one-way ANOVA comparing the biomass of faecal pellets deposited at different locations within *Ranunculus* stands in the Frome / Piddle catchment.

Source	D.F.	SS	MS	F	P
Core	4	17.18	4.3	1.89	0.116
Error	120	272.06	2.27		
Total	124	289.24			

3.3.3 Contribution of faecal pellets to stream material

3.3.3.1 Contribution of faecal pellets to the suspended load

Faecal pellets produced in the Bere Stream had a mean dry weight of 0.1769 mg mm^{-3} (Table 3.28). These data were used to determine the proportion of the suspended load made up of complete faecal pellets. These faecal pellet dry weight data were also used in all subsequent analyses involving the mass of faecal pellets. The proportion of the suspended load at Bere Stream consisting of complete faecal pellets followed a similar pattern to faecal pellet abundance. During March and April the proportions are low (< 0.5 %), as faecal pellet abundance starts to increase the percentage during the summer varies between 1 – 3 %. As peak faecal abundance is reached this rises steeply to 10 % in September and November. It would be expected that the percentage for October would be high, however, on this sampling occasion there was a dramatic increase in the suspended load that rose to 13 mg L^{-1} from around 2 mg L^{-1} in the months immediately

following and preceding it. In general the suspended load data appear uncoupled from faecal pellet abundance (Figure 3.42).

Table 3.28; Calculations of the dry weight of faecal pellets sampled from the Bere Stream

Replicate number	1	2	3	4	5
Vol. of FPs (mm^3)	0.0664	0.0515	0.0457	0.0598	0.0419
Mass of FPs (mg)	0.0151	0.0116	0.0128	0.0116	0.0118
Mass of blanks (mg)	0.004	0.0038	0.0039	0.0034*	0.002
FP mass (mg mm^{-3})	0.1672	0.1516	0.1946	0.1371	0.2338

*** Mean weight of other blanks as aluminium cap torn**

At Baggs Mill the proportion of total suspended load in the form of complete faecal pellets did not reach the same levels as that seen at Bere Stream. There were two peaks of almost 4% in July and November coinciding with that seen in faecal pellet abundance, for the rest of the year the figures remain around 2 %. The suspended load data do not appear to have a strong relationship with faecal pellet abundance (Figure 3.43).

At Maiden Newton the proportion of the suspended load as faecal pellets remained relatively uniform throughout the year. There was no suspended load data for March as the sample was lost in the laboratory, but the proportion of the load as faecal pellets is below 1% for April and May. This rises to between to 1 – 3 % for the rest of the year except for September when it drops to just below 1% (Figure 3.44). There was some variation in the proportions of the stream seston as faecal pellets at East Stoke. Values remained at 1 – 2% over the summer before rising in October and November to over 3 and 4 % respectively and declining again in December back to 2 %. Interestingly this peak in the proportion of suspended load as faecal pellets did not coincide with peak faecal pellet abundance, but slightly preceded it. As the suspended load values increased so the proportion contributed by the faecal pellets decreased (Figure 3.45).

Figure 3.42; Contribution of faecal pellets to Bere Stream suspended load. Bars show the percentage of the suspended load consisting of whole faecal pellets by mass. Points show the suspended load of the water at the time of the sampling time, March 2003 – February 2004. Error bars = 95% confidence intervals.

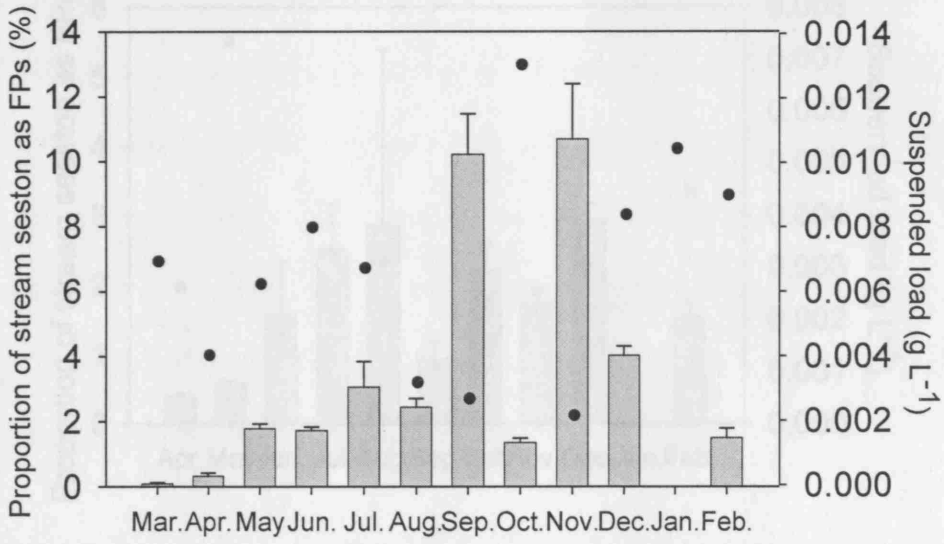


Figure 3.43; Contribution of faecal pellets to Baggs Mill suspended load. Bars show the proportion of the suspended load present as whole pellets and points the suspended load, July 2003 – December 2003. Error bars = 95% confidence intervals.

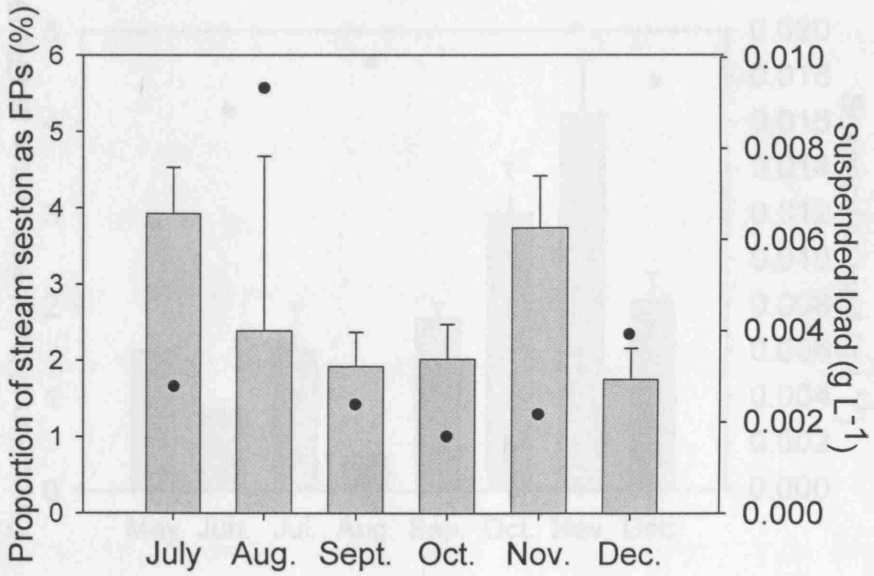


Figure 3.44; Contribution of faecal pellets to Maiden Newton suspended load. Bars show the proportion of the suspended load present as whole pellets and points the suspended load, March 2003 – February 2004. Error bars = 95% confidence intervals.

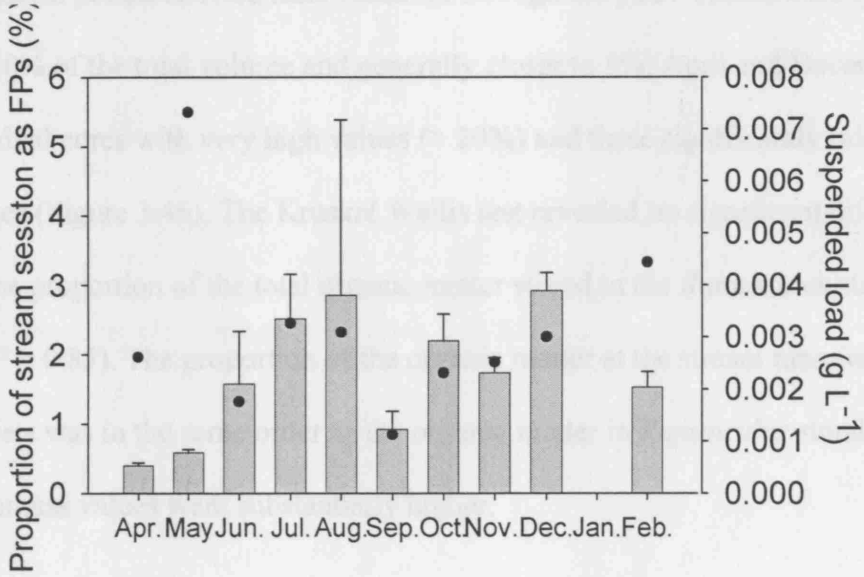
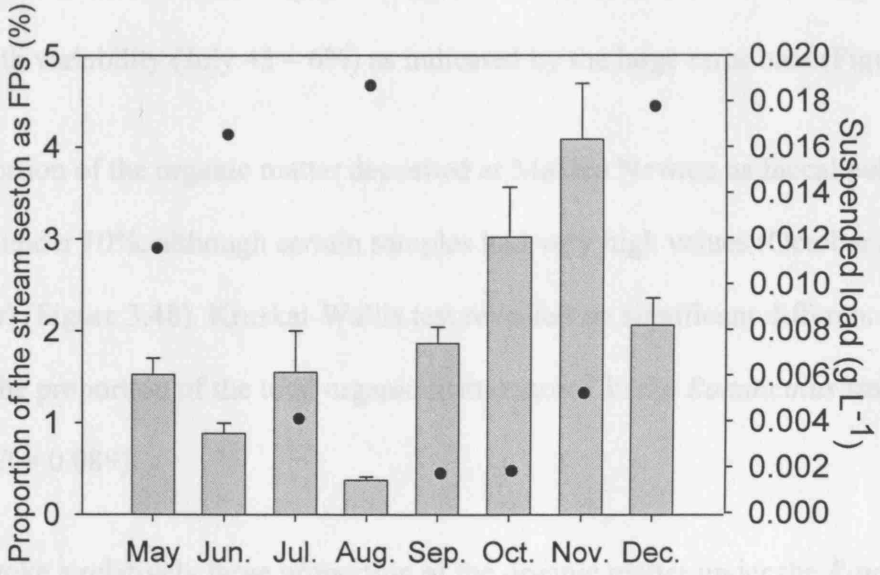


Figure 3.45; Contribution of faecal pellets to East Stoke suspended load. Bars show the proportion of the suspended load present as whole pellets and points the suspended load, May 2003 –December 2003. Error bars = 95% confidence intervals.



3.3.3.2 Contribution of faecal pellets to deposited organic matter

At Bere Stream the proportion of the organic matter trapped within the *Ranunculus* stands as faecal pellets showed little variation through the year. Values were typically less than 10% of the total volume and generally closer to 5%. April and December both had individual cores with very high values (> 20%) and these significantly raised the mean values (Figure 3.46). The Kruskal Wallis test revealed no significant differences between the proportion of the total organic matter stored in the *Ranunculus* stands over the year ($P = 0.85$). The proportion of the organic matter at the stream margins as intact faecal pellets was in the same order as the organic matter in *Ranunculus* stands even though biomass values were substantially higher.

Analysis of the Baggs Mill data using a Kruskal-Wallis test revealed no significant differences between the proportion of the total organic matter stored in the *Ranunculus* stands over the year ($P = 0.117$). There was substantial variation in the proportion of the total organic matter deposited that was in the form of intact faecal pellets. There was substantial inter-month variability (24% July – 0% November) as well as very high intra-month variability (July 43 – 6%) as indicated by the large error bars (Figure 3.47).

The proportion of the organic matter deposited at Maiden Newton as faecal pellets was generally under 10%, although certain samples had very high values (October and December) (Figure 3.48). Kruskal-Wallis test revealed no significant differences between the proportion of the total organic matter stored in the *Ranunculus* stands over the year ($P = 0.089$).

At East Stoke a relatively large proportion of the organic matter under the *Ranunculus* stand consisted of whole faecal pellets with the median values reaching around 20%, with one of the cores in July reaching 87%. The proportions rose from May, when there

were no faecal pellets present to a peak in July. The proportion then declined until again there were no faecal pellets left in the *Ranunculus* stand in October and November before material again accumulated in December (Figure 3.49). A Kruskal-Wallis test revealed no significant differences between the proportion of the total organic matter as faecal pellets stored in the *Ranunculus* stands over the year ($P = 0.558$).

Figure 3.46; The proportion of total organic matter in the form of faecal pellets by volume at Bere Stream. Figure shows monthly percentages under *Ranunculus* stands (A) and variation in marginal deposits (B). Bars are median values and error bars show maximum and minimum values.

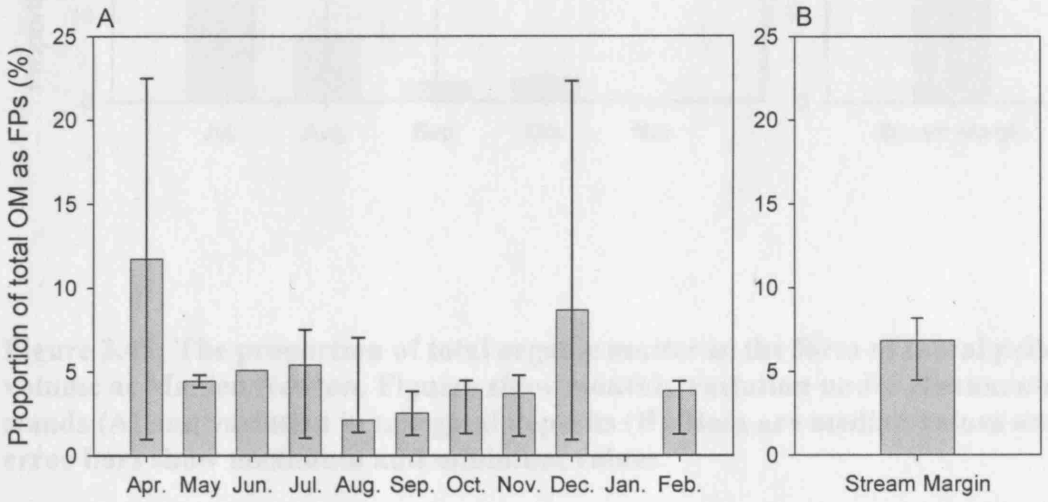


Figure 3.47; The proportion of total organic matter in the form of faecal pellets by volume at Baggs Mill. Figures show monthly variation under *Ranunculus* stands (A) and variation in marginal deposits. July 2003 – February 2004 (all material had been washed out by November 2004) (B). Bars are median values and error bars show maximum and minimum values.

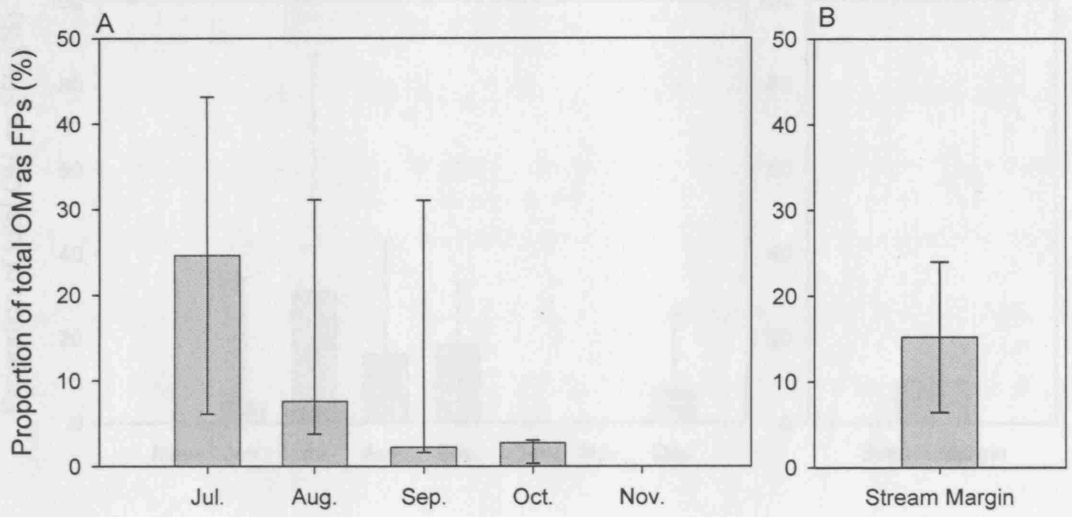


Figure 3.48; The proportion of total organic matter in the form of faecal pellets by volume at Maiden Newton. Figures show monthly variation under *Ranunculus* stands (A) and variation in marginal deposits (B). Bars are median values and error bars show maximum and minimum values.

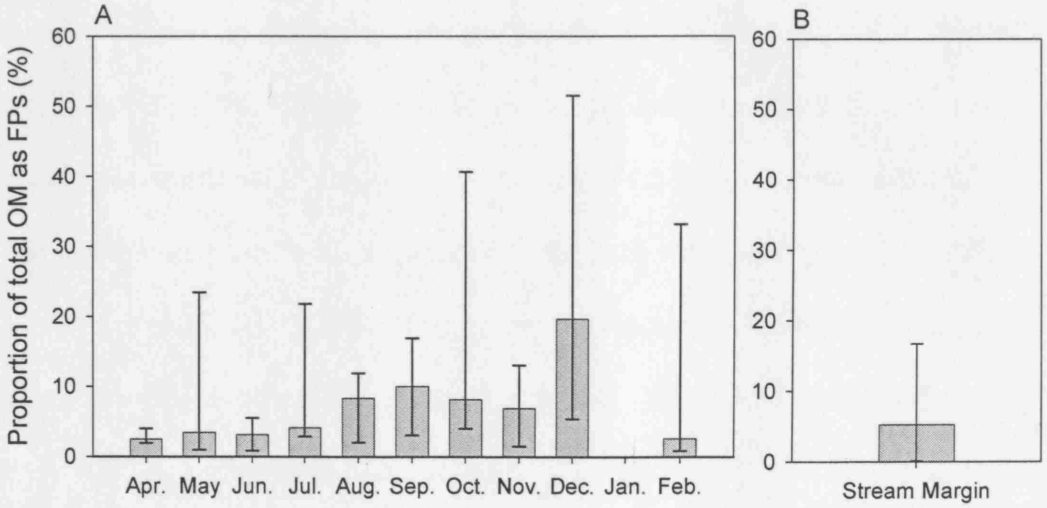
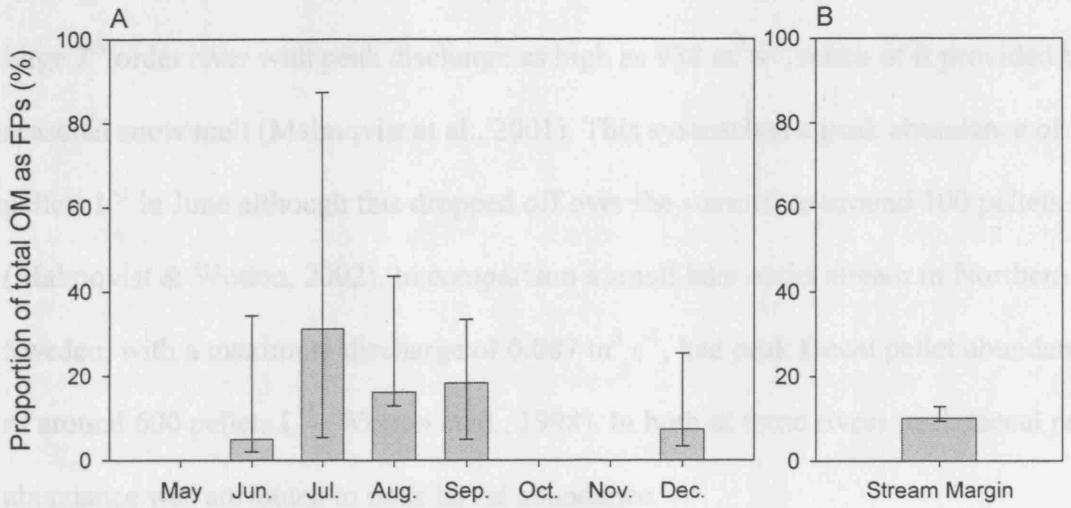


Figure 3.49; The proportion of total organic matter in the form of faecal pellets by volume at East Stoke. Figures show monthly variation under *Ranunculus* stands (A) and variation in marginal deposits (B). Bars are median values and error bars show maximum and minimum values.



3.4 Discussion

There are large numbers of faecal pellets in the water column of these chalk stream systems in comparison to other river systems. The Vindel River in Northern Sweden is a large 7th order river with peak discharge as high as 938 $m^3 s^{-1}$, much of it provided by seasonal snow melt (Malmqvist et al., 2001). This system had a peak abundance of 2000 pellets L^{-1} in June although this dropped off over the summer to around 100 pellets L^{-1} (Malmqvist & Wotton, 2002). In comparison a small lake outlet stream in Northern Sweden, with a maximum discharge of 0.087 $m^3 s^{-1}$, had peak faecal pellet abundance of around 600 pellets L^{-1} (Wotton et al., 1998). In both of these rivers peak faecal pellet abundance was attributed to peak larval abundance.

In the Frome / Piddle catchment faecal pellet abundance can reach a maximum peak of almost 10,000 pellets L^{-1}, while values of >3000 pellets L^{-1} are not uncommon. The lowest value recorded was a mean of 236 pellets L^{-1} from the Bere Stream in March 2003, although, in general, numbers were typically >500 pellets L^{-1}. Despite these large differences in faecal pellet numbers the peak blackfly larvae population sizes in the three rivers are of a similar order of magnitude, 10^5 larvae m^{-2} (Ladle et al., 1972; Wotton et al., 1998; Malmqvist et al., 2001). However, the population at the lake outlet stream was restricted to a very short reach while the figure for the large northern snowmelt river refers to the population found in the rapids (Wotton et al., 1998; Malmqvist et al., 2001). In contrast the populations in the Frome / Piddle catchment is found attached to *Ranunculus* plants (Ladle et al., 1972), which themselves occupy up to 76% of the surface area of the channel (Wharton et al., 2006) over extensive reaches of the channel. Thus, total blackfly larvae populations per unit discharge in the Frome and Piddle may exceed those in the other two systems. An additional factor that may be responsible in part for the high abundance of faecal pellets in these systems is the

location of the larvae. In chalk streams they are high in the water column attached to *Ranunculus* stems. Pellets released in chalk streams will enter transport high in the water column compared to the other two rivers where larvae are attached to the stream substratum, thereby increasing the likelihood of recently released pellets becoming trapped.

Chalk streams clearly exhibit a different pattern of annual variation in faecal pellet abundance. Comparatively low levels of faecal pellets are present in the summer when larval populations are at their highest, while the highest pellet levels are in the winter period when larval populations are at both a seasonally low level and in an over wintering state. This suggests high rates of faecal pellet sedimentation and storage over the spring and summer period of peak larval abundance, and a seasonal flushing event of this material from the river in the winter. The flushing of large deposits of faecal pellets as flows increase has been hypothesised (Wotton et al., 1998), however, it would appear that this is the first time that this process has been recorded. The relationship between faecal pellet abundance and environmental factors such as macrophyte cover and discharge is not explicit and it is likely that there is not just one factor controlling abundance. In addition, it is possible that areal *Ranunculus* cover is not the most significant control of faecal pellet abundance and that plant biomass is of more importance.

It has been noted that the traditional management practises of weed cutting in chalk streams leads to the release of large quantities of fine sediments (Sand-Jensen et al., 1989; Wood & Armitage, 1999) and the seasonal dieback of macrophytes has similar effects (Sand-Jensen et al., 1989; Wood & Armitage, 1997). Therefore it is probable

that if macrophytes act as a substantial store of faecal pellets then their seasonal growth cycle may impact on faecal pellet dynamics in these systems.

The only differences in faecal pellet abundance upstream and downstream of a *Ranunculus* stand were found at East Stoke. *Ranunculus* stands are restricted to areas with suitable light levels for growth and are typically part of a larger macrophyte-rich reach. As blackfly larvae are found in association with macrophytes then there will be large numbers of faecal pellets being produced and transported in these reaches. These adjacent stands will probably override any affects the presence of individual *Ranunculus* stands may have on faecal pellet abundance. In order to see the impacts of *Ranunculus* on faecal pellets it may be necessary to look at the larger reach scale.

Comparing sites from upstream to downstream in the Frome / Piddle catchment showed no clear patterns in faecal pellet abundance, with virtually all the sites being highly significantly different from the other sites within that river system. This suggests that faecal pellet abundance is a local phenomenon and not part of larger catchment-wide processes. This pattern is in contrast to large northern rivers where there is an increase in faecal pellet abundance with increased distance downstream, along with a positive relationship with stream discharge (Malmqvist et al., 2001) and stream size (Malmqvist & Wotton, 2002). These river systems have a highly dynamic discharge regime dominated by snowmelt and contain virtually no rooted macrophytes (Wotton pers. comm.), in complete contrast to the chalk streams studied in this study. The pattern of faecal pellet abundance was reversed in the lake-outlet river, there was a decline in the abundance of faecal pellets with distance downstream, due to the majority of the larval blackfly population being present at the upstream end of the stream (Wotton et al., 1998). Losses of particles from the water column generally follow an exponential decay

model (Reynolds et al., 1990), explaining, in part, the downstream decrease in faecal pellets. Mid-way through the study reach there was an increase in the number of pellets deposited, coupled with a decrease in the numbers in transport (Wotton et al., 1998), indicating areas conducive to faecal pellet sedimentation.

Sites promoting particle deposition impact greatly on the transport of faecal pellets. On the River Vindel the lowest faecal pellet abundance was found in a slow flowing section of the river (Malmqvist & Wotton, 2002). Over a total sampling distance of 180 km a slow flowing reach of just 8 km was able to reduce the number of faecal pellets in transport by up to 90% (Malmqvist et al., 2001). Previous studies on faecal pellet abundance showing a positive relationship between pellet abundance and discharge were attributed to lower sedimentation rates at higher discharges (Malmqvist et al., 2001). This relationship was not so strong in the present study probably as a result of the influence of rooted macrophytes that exert a strong influence on sediment dynamics in chalk streams (Sand-Jensen, 1998).

There are clear differences in the volume of faecal pellets found at different locations within the stream; pellets in transport were significantly smaller than those deposited within *Ranunculus* stands and at the stream margin. This is to be expected as there is a positive relationship between the volume of a pellet and its fall velocity (Malmqvist et al., 2001; Giles & Pilditch, 2004) and larger pellets have been shown to be preferentially lost over small pellets along a small lake outlet stream (Wotton et al., 1998).

There has been increasing interest in the horizontal flux of faecal pellets in streams and rivers (Wotton et al., 1998; Malmqvist et al., 2001; Malmqvist & Wotton, 2002) in contrast to the vertical fluxes present in marine environments. However, these data on

the volumes of faecal pellets at different locations within the stream show there are also strong vertical linkages within these lotic systems as large pellets are rapidly transferred from the water column to the substratum. The dimensions of the average pellet in suspension is in the order of 75 x 65 μm, while those deposited in *Ranunculus* stands and at the stream margin are around 180 x 170 μm. This transfer of material by blackfly larvae acts to enhance pelagic-benthic coupling within the stream environment through the phenomena of spiralling as described by Wallace et al. (1977).

The data on the abundance of faecal pellets occurring through the river systems and the volumes of faecal pellets at different locations within a site suggest that these are highly retentive systems that have areas capable of trapping and storing large quantities of faecal pellets and other particles. The partitioning of pellets in the stream may also explain why faecal pellets make up a small proportion of the suspended load in comparison to the Vindel River. The largest pellets would contribute most to the suspended load but are deposited within the stream.

The data on the volumes of faecal pellets in suspension and their distribution over the year showed a complicated picture as might be expected. However, a clear pattern can be seen for February, which has faecal pellets with the largest volumes in transport. This occurs for all of the sites and for the catchment as a whole providing further evidence for the winter flushing event. The primary determinant on the initiation of transport for particles at a given flow is the size of the particle (Knighton, 1998; Sand-Jensen, 1998). Therefore for discreet particles such as faecal pellets increasing flows are capable of transporting larger particles. As flows increase in chalk streams the smallest pellets are successively winnowed out of the deposits until only the larger flows that

occur in winter, after the aquifer has recharged, will be capable of transporting the largest pellets downstream.

Significant numbers of faecal pellets were deposited within the *Ranunculus* stands. Macrophyte stands act as efficient traps for faecal pellets and other fine particles as flows are rapidly attenuated as they enter the stands (Sand-Jensen, 1998). This is analogous to the increased deposition of fine particles seen with extensive mussel beds where as flows enter the matrix formed by the shells of the mussels velocity is decreased and deposition enhanced (Ragnarsson & Raffaelli, 1999). The rhizome and root structures of the *Ranunculus* will act to increase the stability of the underlying stream substratum (Sand-Jensen et al., 1989; Fritz & Feminella, 2003), presumably increasing the retention of organic particles deposited above this layer. *Ranunculus* stands showed no clear patterns of distribution for either faecal pellet biomass or faecal pellet volume within the stand. This may be due to the *Ranunculus* stand structure offering protection to the pellets from the full winnowing effects of the flow (Sand-Jensen et al., 1989; Sand-Jensen, 1998), thereby reducing the size selective sorting of the pellets.

Organic matter deposits at the stream margins represent areas of high faecal pellet abundance, being of a substantially higher magnitude than those found within *Ranunculus* stands. These deposits are found in both straight reaches and on the inside of channel meanders and are analogous to the channel features of margin and point bars formed by inorganic particle deposition as a result of the decline in flow velocity in these regions (Wood & Armitage, 1997; Knighton, 1998; Wood & Armitage, 1999). Marginal deposition is further enhanced by riparian vegetation encroaching into the channel and the development of macrophytes, particularly *Apium nodiflorum* and

Rorippa nasturtium-aquaticum, both of which characteristically grow from the channel margins inwards. Studies on other lowland streams have found marginal areas to be efficient trapping areas for organic particles although the quantities of material deposited was low in comparison to macrophytes as increases in water levels promoted resuspension and export (Wanner & Pusch, 2001). Within chalk streams the stable flow regimes may have resulted in the high biomass values as there may be few resuspension events so allowing steady accumulation of the pellets.

The data presented for faecal pellets in this system are considered a conservative estimate of blackfly mediated aggregation as a particle was only counted as a faecal pellet if it was intact. The distinctive shape of blackfly faecal pellets are one of the main determinants that separate them from faecal pellets produced by other macroinvertebrates (Ladle & Griffiths, 1980). However, many of the other particles appeared to have been pellets originally but had subsequently started to become disrupted. Thus the impact of faecal pellets on the organic matter of the Frome and Piddle may be greater then these data suggest. There are few references describing the breakdown of faecal pellets although Taghon et al. (1984) describes the breakdown process through friction as a faecal pellet is transported along the substratum of a flume. This described parts of the pellets breaking away from the main structure. Many of the particles seen in the Frome and Piddle had the fine particle matrix seen as a result of filter-feeding activity, but not the distinctive blackfly faecal pellet shape. These particles may have formerly been part of larger pellets that have undergone the breakdown process. As the particles were reduced in size Taghon et al. (1984) also described the smaller particles passing an entrainment threshold and suspended transport being initiated, demonstrating the size selective pressures of flow, and suggesting that

deposited particles within the stream may be resuspended and undergo further downstream transport as disaggregation processes occur.

The capture, transformation and transfer of material from the water column to the stream substratum will cause changes to the stream environment. In marine environments biodeposits either increase the erodibility of the substratum by increasing the particle size of the sediments or decrease the erosion threshold as the cohesive effects of fine sediments are lost as they are incorporated into larger faecal pellets (Taghon et al., 1984; Willows et al., 1998; Drake et al., 2002; Giles & Pilditch, 2004). Within chalk streams faecal pellet production is predicted to decrease the erodibility of the fine sediments as the fine inorganic sediments that form cohesive substrata (particle size < 4 μm) are found at very low quantities in the Frome / Piddle, typically less than 1% (Wharton et al., 2006). Thus the effect of faecal pellet production is to increase particle size and so decrease the transport potential of this material. It is likely that much of the organic material that is transferred to the substratum would have been lost to the system had the larvae not incorporated it into the larger faecal pellets that then undergo the vertical transfer to the substratum (Ladle & Griffiths, 1980).

These deposited pellets become a detritus resource for utilisation by the stream community. Macroinvertebrates use deposited pellets as both a food resource and in the production of tubes by midge larvae (Ladle & Griffiths, 1980; Wotton et al., 1998). Structures within streams that promote the trapping of organic particles become sites of high secondary production as the microbial community supported by these organic deposits form a food resource for macroinvertebrates (Edwards & Meyer, 1990). It may be that the production of faecal pellets produces stable organic deposits that fulfil a comparable role in chalk streams.

Faecal pellet production will lead to changes in biogeochemical cycling in streams as dissolved organic carbon and nitrogen is captured and bound into pellets. Their deposition in large numbers at the stream margin may act as a source of fertiliser for marginal and riparian vegetation (Malmqvist et al., 2004). Bivalve aggregations, in both marine and freshwater systems, cause localised enhancement of carbon and nitrogen in surrounding sediments due to the presence of extensive biodeposits (Strayer et al., 1999; Norkko et al., 2001; Vaughn et al., 2004). Biodeposits thus cause changes in the absolute quantities of nutrients in the sediments but it is also hypothesised that they alter the release of these substances to the environment by acting as a time-release capsule as the rate of release is much lower then that for the smaller constituent particles (Drake et al., 2002).

4 Impacts of *Simulium* on the modification and transport of stream seston

4.1 Introduction

Blackfly larvae are found at high population densities and are known to produce large numbers of faecal pellets (Ladle et al., 1972; Wotton, 1987; Wotton et al., 1998; Malmqvist et al., 2001). The production of pellets by filter feeders involves the transformation of a large number of small particles into fewer, but much larger particles. Blackfly larvae are capable of ingesting dissolved organic matter (Hershey et al., 1996), therefore their pellets can be 5 x 10^8 times larger than their smallest particles (Warren et al., 2004). Studies have shown that, for the finest particles, incorporation into pellets increases their fall velocity by up to two orders of magnitude (Drake et al., 2002). This transformation of particles has the potential to significantly alter organic matter dynamics in rivers and influence the downstream dispersal of this material.

Other filter feeders, such as mussels, also produce large numbers of pellets, in the form of faeces and pseudofaeces (particles rejected for ingestion that are bound in mucus and released back into the environment). These pellets act as an important mechanism by which pelagic particles are transferred to the substratum in marine environments (Norkko et al., 2001; Giles & Pilditch, 2004). Areas of deposited pellets are associated with changes to the environment. Pellets mediate the transport dynamics of sediments, either increasing or decreasing the sediment erosion thresholds dependent on substratum characteristics (Drake et al., 2002; Giles & Pilditch, 2004). They can also alter the biogeochemical status of sediments by increasing their carbon and nitrogen concentrations and by the slow release of molecules bound in the pellets (Willows et al., 1998; Norkko et al., 2001; Drake et al., 2002).

The impacts of filter feeders on freshwater ecosystems has previously received limited attention (Strayer et al., 1999). Studies have shown that high population densities of bivalves have dramatic impacts on rivers. Zebra mussels (*Dreissena polymorpha* Pallas) remove significant quantities of particles from the water column and transfer these to the substratum. Phytoplankton production and respiration was reduced in a French / German river as zebra mussel population densities increased. The transformation of this suspended material changed the location of organic matter processing within the river. Processing was decreased in the water column and increased on the substratum as the filtered material was transformed into pellets and transferred to the substratum (Descy et al., 2003). Studies on the Hudson River, USA, found that bivalve-mediated organic-rich deposits created a carbon flux of 3 g m^{-2} d^{-1}, causing changes in consumer populations and increases in microheterotrophic production and biomass (Strayer et al., 1999), presumably as a result in changes to resource availability.

Blackfly larvae increase the retention of particles in lake outlet streams. Dense larval aggregations can remove 0.8 – 1.4 % of a streams particulate load per longitudinal meter of stream length (Morin et al., 1998), demonstrating the potential for substantial transformation of the stream seston. Another study on a small, shallow, lake outlet concluded that approximately one third of the pellets produced by a dense larval aggregation were retained within the stream. This created a carbon flux of particles from the water column to the stream substratum of 2.9 g m^{-2} d^{-1} (Wotton et al., 1998), similar to values obtained for zebra mussels (see above).

Particle characteristics are a key determinant of particulate transport in streams. Releases of natural organic particles in both natural and artificial channels have shown an inverse relationship between increasing particle size and the distance travelled before

deposition (Webster et al., 1987). Field studies on factors influencing the deposition of fine particles in streams have found that for very fine particulate organic matter (15 – 52 μm) gravitational force plays a minimal role in the deposition of particles to the stream bed. However, as particles increased in size to > 50 μm, (to a maximum of 250 μm in this study) then gravitational effects had a stronger influence on the deposition of particles (Thomas et al., 2001). Transformation into blackfly faecal pellets (up to 400 μm long) will influence particulate dynamics as the constituent particles of the pellets will frequently be in the size range unaffected by gravitational force until bound into a pellet where gravitational force can then exert an influence on their fate.

In addition to the effects of particle size on fall velocity the density of a particle has a strong influence on its transport; as density increases, the fall velocity of the particles increases correspondingly. Faecal pellet density is controlled primarily by the density of the ingested constituent particles. High quantities of mineral particles in the diet result in higher fall velocities as the constituent particles of the pellets have a higher density (Giles & Pilditch, 2004). Interspecific differences in the formation of the pellets will lead to variations in fall velocity and the density of packing of the constituent particles. Less tightly packed pellets have more fluid-filled pore space then tightly packed particles and thus a lower density (Taghon et al., 1984; Ladle et al., 1987).

This study investigated the influence of blackfly larvae on the size range of particles in the water column. It is hypothesised that the feeding behaviour of blackfly larvae will alter the size range of particles within the water column. Particles ingested by the larvae will be aggregated into the larger faecal pellets, egested back into the stream environment leading to a shift in the size class of the particles towards larger sizes. The size and density of blackfly faecal pellets will partially determine the transport potential

of the pellets. Larger pellets will be deposited at faster rates then smaller pellets. Pellets of equivalent size will only show differences in their transport in relation to differences in the density of the constituent particles.

It is hypothesised that differences in the density of the food ingested will lead to differences in the fall velocity of the pellets produced. Mineral particles have a higher density then organic particles; thus pellets produced from a diet high in organic particles will be transported a greater distance then those produced from mineral particles. Seasonal variation in the density of material transported by a stream will cause variation in the transport of pellets. Pellets will be transported further in the summer as organic material from autochthonous production increases, and that as higher winter discharges enable the entrainment and transportation of mineral particles then pellet transportation will decrease.

As pellets age they increase in size and therefore porosity (see chapter 6). An increase in porosity will increase the proportion of fluid in the pellets and the pellet density will decrease. Older pellets of a given size are therefore predicted to travel further then younger, freshly-produced pellets.

4.2 Methods

Blackfly larvae were maintained in laboratory microcosms to determine their effect on the size range of particles in the water column. The Microcosms consisted of plastic buckets, each containing 8 L of natural stream water. Water was circulated by a continuous stream of bubbles produced from an airstone on the base of the bucket, thereby mimicking the flowing environments found in chalk streams. The water for these experiments was collected from the River Chess, providing a local source for the large quantities of water needed.

The quantity of particles contained in the water was determined for a series of size classes under the following treatments:

(a) natural stream water

(b) natural stream water through which a stream of bubbles had been passed using an airstone and an aquarium pump. This controlled for the effects of the bubbles on particle size.

(c) as in (b) but with the addition of blackfly larvae to modify particle size range

Blackfly larvae were acclimatised to laboratory conditions in a central holding tank of bubbling water. Following acclimatisation larvae were carefully transferred to each experiment using a disposable plastic Pasteur pipette. An average of 10 larvae were removed on each occasion and transfer continued until approximately 800 larvae had been added to each bucket. Larvae of varying sizes were added to ensure that the influences on particulate size were similar to that encountered in natural systems as water flowed over of larvae mixed size. The two recirculating treatments were left for 24 h to provide the larvae with enough time to modify the particles in the water.

At the end of each experiment, water in the buckets was stirred to suspend all particles and poured through a 500 μm sieve into another bucket (the majority of larvae remained either attached to the wall of the bucket or were retained on the sieve). Any larvae remaining in the samples were removed using watchmaker's forceps.

The size range of particles in the water was determined by passing the sample through a series of mesh nets and sieves to separate out fractions. Three sieves of 212 μm, 106 μm and 63 μm pore size were used, as well as a 25 μm Monyl mesh net and 1.6 μm Whatman GF/A glass microfiber filters. The separation point between the size classes was similar to that used by Hershey et al. (1996). At each separation stage the sample was passed through the filter/sieve, rinsed with distilled water thereby ensuring no contamination with solutes, and the elutriant washed on to a pre-dried and pre-weighed Whatman GF/A glass microfiber filter. These were dried overnight at 80°C (J. Cotton, pers. comm.) and re-weighed to give the dry mass of particles contained in each size class.

Measures of the transport capabilities of particles have typically focused on the fall velocity or rate at which a particle settles through a quiescent water column (Dietrich, 1982; Taghon et al., 1984; Ladle et al., 1987; Wotton et al., 1998; Giles & Pilditch, 2004). Recently there has been some debate as to how laboratory derived fall velocities relate to values of depositional velocities obtained under natural conditions (Hall et al., 1996; Minshall et al., 2000). Thomas et al. (2001) found that as particles increased in size gravitational effects became increasingly important and calculated fall velocity values were correlated with the deposition velocity of particles. The suggested threshold for gravitational influence was given as ~ 50 – 100 μm (Thomas et al., 2001), interestingly the work of Hall et al. (1996) and Minshall et al. (2000) was based on

particles of 2 and 53 – 106 μm respectively and may therefore be too small for gravitational influences to be important and for fall velocities to relate to depositional velocities. The smallest pellets that could realistically be manipulated and tracked in this study were those larger than 50 μm and therefore measured fall velocities were considered to be a good predictor of particle transport.

It was necessary to maintain blackfly larvae in the laboratory to provide enough pellets for still water fall velocities. Laboratory production provided control of the age of the pellets and the diet from which they were produced allowing the role of these factors on pellet fall velocity to be determined. Identical procedures were maintained for all experiments. Stream water was collected in plastic carboys with a volume of between 8 – 12 L; the carboys were first rinsed out with the stream water and then re-filled with the water to be taken back to the laboratory, where the water was kept at 4°C to reduce biotic activity prior to use (Minshall et al., 2000).

Larvae were collected by wading into the stream to locate dense aggregations that were typically located on the trailing leaves of *Ranunculus* plants, notably those in the fastest flow. During the late summer, as flows decreased in both volume and velocity, many of the *Ranunculus* stands developed a thick covering of algae. This reduced the number of larvae attached to the plants and so peak larvae abundance was found in shaded areas, i.e. from overhanging riparian vegetation, which prevented epiphytic growth. Leaves colonised by large numbers of larvae were removed from *Ranunculus* plants and placed in plastic bags, with a minimum of water left in the bag to ensure a high survival rate of larvae (J.A.B. Bass, in pers. comm.). After return to the laboratory, *Ranunculus* leaves with attached larvae, were placed in 6 L Perspex tanks filled with stream water.

Vigorous water movement, essential for larval survival, was maintained by circulating water using aquarium pumps and air stones.

To produce pellets for experiments, c.50 larvae, of varying sizes, were transferred to a 3L Pyrex conical flask. To represent the variation in pellet volumes found in streams the larvae added were of different sizes so producing pellets of varying volume. The flask was filled with the stream water and an air stone inserted to produce a current. To generate the large number of pellets needed for the experiment to determine the effect of age on pellet fall velocities, a scaled-up version of the technique described above was used, with two 8L buckets substituted for the conical flasks.

The water added to the flasks contained particles of differing origins to determine the effects of varying diet on pellet transport. Literature values for gut throughput time of blackfly larvae typically fall between 20 mins to 1 hr (Ladle et al., 1972; Wotton, 1978), therefore larvae were left for approximately one and a half hours to allow them to feed on the new food source and to complete the egestion of pre-experimental material.

At the end of this period the conical flask was vigorously swirled to suspend the particles and all water and particles poured away. The flask was then refilled with the same stream water ensuring that pellets produced for the experiments consist entirely of the material contained in the water as all material ingested prior to the experiment will have been excreted and replaced with material from the added water. Each experiment was left overnight to allow larvae to produce enough pellets to determine their fall velocity. Pellets were harvested by swirling the conical flask so that any particles on the bottom were re-suspended. The contents were then poured through a 500 μm sieve to remove larvae and particles retained by passing the water through a 25 μm Monyl mesh net. Using a wash bottle containing tap water, pellets were transferred into a glass petri

dish and kept at room temperature prior to experimentation for the determination of fall velocities.

The method of Wotton et al. (1998) was used to measure the fall velocity of individual pellets. A 50 ml graduated cylinder was filled with tap water and allowed to equilibrate with room temperature, previous studies have demonstrated that the range of temperatures found within the laboratory would have no significant difference on the fall velocity of the blackfly faecal pellets (Ladle et al., 1987), therefore fall velocity values obtained in the laboratory would be expected to be similar to those obtained at field temperatures. Individual pellets were measured under a dissecting microscope using a calibrated eyepiece micrometer. Each pellet was then collected using a Pasteur pipette and released under the surface of the water in the cylinder, falling at least 10 cm to reach terminal velocity (Ladle et al., 1987). The time taken for the pellet to fall the next 5.5 cm (Wotton et al., 1998) was then measured. A total of thirty pellets, of varying volumes, were released for each treatment.

To determine the factors that influence the fall velocity of pellets, three different experiments were conducted to investigate the role of diet and age on the transport of pellets in streams. To determine if the fall velocity of pellets demonstrated any annual variation due to differences in the particulate load of the streams, larvae were maintained in stream water collected at 3 monthly intervals from April 2004 to January 2005, from Tadnoll Brook. Tadnoll Brook drains heathland, the fast draining nature of heathlands subjects this reach to a stronger influence from local climatic events than a more typical chalk stream draining a chalk aquifer. This will lead to a flashier flow regime and provide a greater opportunity to determine any annual variability.

The effects of diet on sinking rates were investigated by varying the proportions of organic and mineral material in the water. The stream water, organic and inorganic matter used in the experiments were all collected from the River Chess as this provided a locally available source of material. A bank soil sample taken adjacent to the River Chess was chosen to provide mineral particles, as this would most closely match the mineral material that enters the stream. The organic material consisted of equal quantities of *Ranunculus* and Alder *Alnus glutinosa* (L.) Gaertner. A total of five treatments were used for the experiment:

(a) **Natural** - Fresh stream water

(b) **Mineral only** - Stream water to which mineral particles were added

(c) **Organic dominated** - stream water containing 33% mineral particles and 66% organic particles

(d) **Mineral dominated** - stream water containing 66% mineral particles and 33% organic particles

(e) **Organic only** - stream water to which organic particles were added

To provide the organic and inorganic material in a form that the larvae could ingest the leaves and soil samples were dried overnight at 80°C, placed in a blender and passed through a 25 μm net. The material for treatments 2 – 5 were added to natural stream water that had been filtered through 1.2 μm Whatman GF/C glass microfiber filters to remove most particles. The division between particulate organic matter and dissolved organic matter is conventionally set at 0.45μm, however due to the filters blocking it was necessary to use the 1.2 μm division as this removed the majority of particles whilst allowing efficient filtering of the water.

To determine whether conditioning of faecal pellets influenced their fall velocity, pellets were placed in filtered stream water and incubated at 15°C for 41 days. At this stage the pellets had begun to bind together and the manipulation and measurement of individual pellets was no longer possible. Pellets were subsampled on days, 0, 10, 17, 27, 31 and 41 using a Pasteur pipette. Measurements of fall velocities were taken approximately weekly, however due to other research activities it was not possible evenly space the sampling period. The subsampled pellets were transferred to a glass petri dish containing tap water and their fall velocities calculated as above.

4.3 Results

4.3.1 Influence of blackfly larvae on the particle size distribution of stream seston

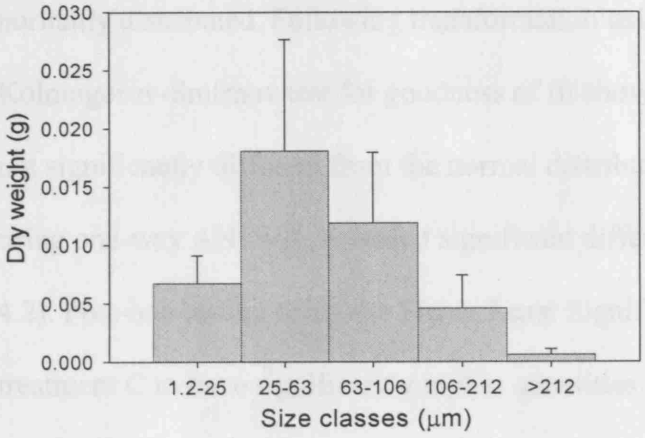
The three treatments showed differences in the quantity of particles contained within each of the size classes. The particle size distribution is unimodal in both normal stream water and bubbled stream water, this changes to a bimodal distribution for the bubbled stream water with larvae (Figure 4.1). Stream water that had blackfly larvae present showed an increase in the quantity of particles in the size classes 106 - 212 μm and > 212 μm of between one and two orders of magnitude in relation to normal and bubbled stream water. Additionally, the quantity of particles in the smallest size class, 1.2 - 25 μm, was elevated in the blackfly treatment compared to the other treatments (Figure 4.1, Table 4.1). Normal stream water had the lowest values for particles in the > 212 μm size class, while bubbled stream water exhibited elevated quantities in both the largest size class (> 212 μm), and the smallest (> 1.2 - < 25 μm), compared to normal stream water. When the quantities of particles were summed to give total particulate mass then the treatment containing blackfly larvae had almost twice the total quantity of particles than the other two treatments (Table 4.1).

Table 4.1; Mean mass of particles size classes and total particles in treatments, A; Normal stream water, B; Bubbled stream water, C; Bubbled stream water and larvae (n = 3).

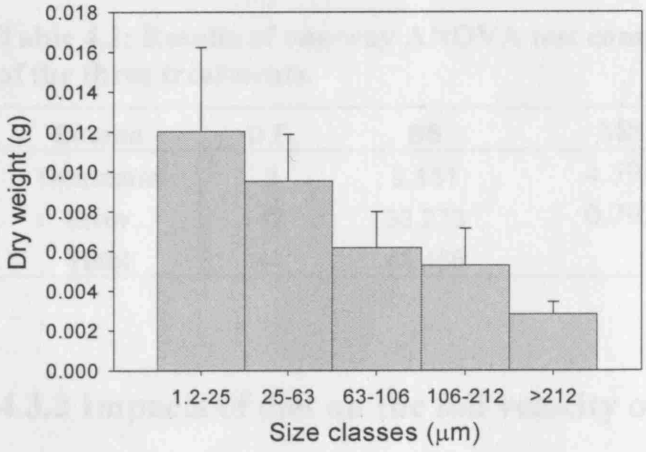
Particulate size range (μm)	Mean mass (g) Treatment A	Treatment B	Treatment C
> 212	0.00063	0.00282	0.01370
< 212 - > 106	0.00459	0.00529	0.01880
< 106 - > 63	0.01192	0.00618	0.00682
< 63 - > 25	0.0181	0.00952	0.01247
< 25 - > 1.2	0.00675	0.01201	0.02554
Total Particles (g)	0.04198	0.03582	0.07733

Figure 4.1; Mean particulate size class distribution of stream water under three treatments, (a) natural stream water, (b) bubbled natural stream water, (c) bubbled natural stream water and blackfly larvae (n = 3) Error bars = 1 standard deviation.

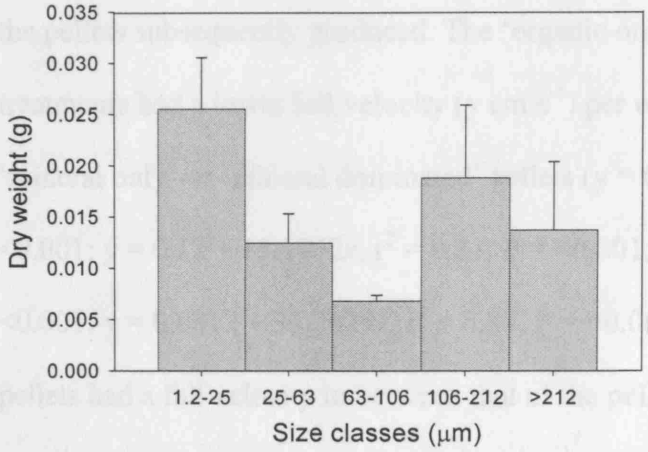
a)



b)



c)



The data were statistically analysed using one-way ANOVA. This test requires the data to be normally distributed and the distribution of the data was tested using the Kolmogorov-Smirnov test for goodness of fit, this showed that the data were not normally distributed. Following transformation using natural logarithms the Kolmogorov-Smirnov test for goodness of fit showed that these transformed data were not significantly different from the normal distribution (P = 0.082). Statistical analyses, using one-way ANOVA, revealed significant differences between the treatments (Table 4.2). Post-hoc testing using the Fisher Least Significant Difference (LSD) test showed treatment C to have significantly higher quantities of particles in the larger size classes (P = < 0.05).

Table 4.2; Results of one-way ANOVA test comparing the particulate size classes of the three treatments.

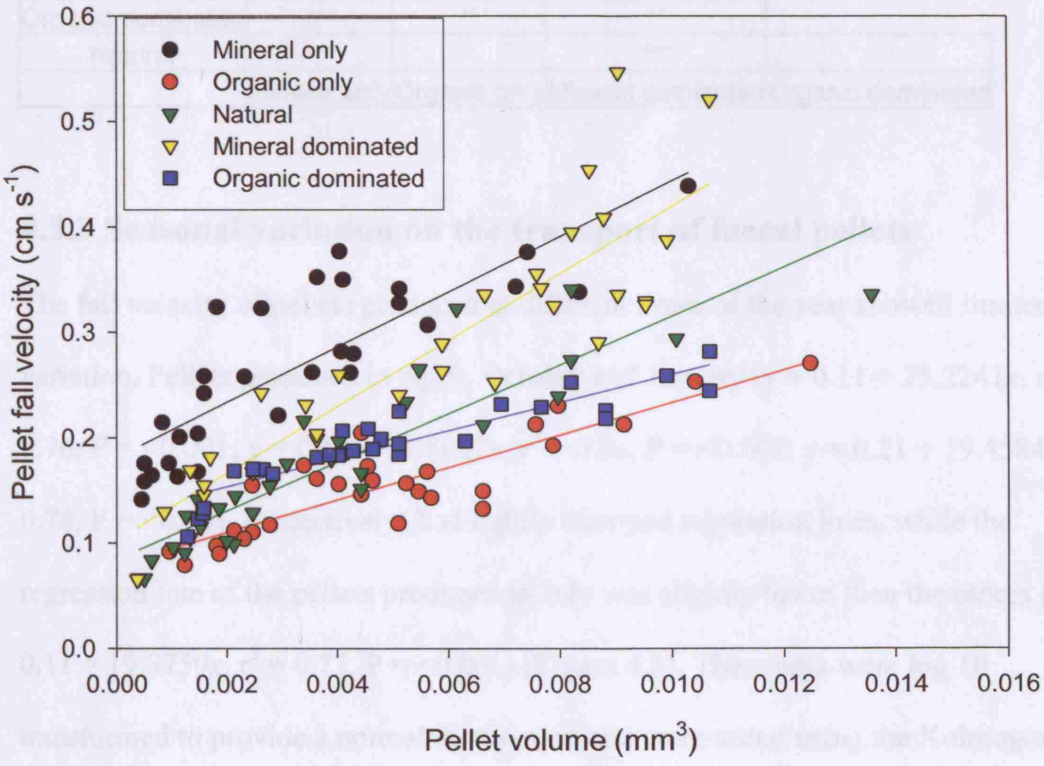
Source	D.F.	SS	MS	F	P
Treatment	2	9.181	4.591	5.79	0.006
Error	42	33.273	0.792		
Total	44	42.455			

4.3.2 Impacts of diet on the fall velocity of faecal pellets

The type of particles fed to blackfly larvae had a significant affect on the fall velocity of the pellets subsequently produced. The 'organic only' and 'organic dominated' treatments had a lower fall velocity (y cm s^{-1}) per unit volume (x mm^3) then either the 'mineral only' or 'mineral dominated' pellets ($y = 0.07 + 15.5385x$, $r^2 = 0.75$, P = <0.001; $y = 0.12 + 15.1862x$, $r^2 = 0.83$, P = <0.001; $y = 0.16 + 30.4504x$, $r^2 = 0.75$, P = <0.001; $y = 0.0812 + 33.2629x$, $r^2 = 0.88$, P = <0.001, respectively). The 'natural' pellets had a fall velocity in between that of the pellets produced from the organic and mineral diets ($y = 0.07 + 25.9281x$, $r^2 = 0.85$, P = <0.001) (Figure 4.2).

The data were tested for normal distribution using the Kolmogorov-Smirnov test for goodness of fit (see above). This showed these data not to be normally distributed, after log 10 transformation they conformed to the requirement for normal distribution (P = 0.126). ANCOVA showed highly significant differences between the fall velocities of pellets produced after feeding larvae on different diets (Table 4.3).

Figure 4.2; Plots of the fall velocity of the pellets produced on the five diets containing differing quantities of organic and inorganic material in relation to their volume.



Tukey's *post-hoc* test showed that the fall velocity of 'natural' pellets was not significantly different from the 'organic only' or 'organic dominated' pellets. 'Natural' pellets did have significantly different fall velocities from 'mineral only' and mineral dominated' pellets. The two mineral treatments did not have significantly different fall velocities, although there was a significant difference between the fall velocities of pellets produced from the 'organic only' and 'organic dominated' diets (Table 4.4).

Table 4.3; Results of ANCOVA comparing the fall velocity of pellets produced after feeding the larvae different diets.

Source	D.F.	Seq SS	Adj SS	Adj MS	F	P
Volume	1	3.4158	3.697	3.697	235.7	0.000
Diet	4	1.6852	1.6852	0.4213	26.86	0.000
Error	146	2.2901	2.2901	0.157		
Total	151	7.3911				

Table 4.4; Results of *post-hoc* Tukey test showing significant differences between the fall velocity of pellets produced from larvae fed different diets. Asterisks represent P values, * P = <0.05, ** P = <0.01, *** P = < 0.001.

Organic only	***			
Mineral dominated		***		
Organic dominated	***	**	**	
Natural	***		***	
	Mineral only	Organic only	Mineral dominated	Organic dominated

4.3.3 Seasonal variation on the transport of faecal pellets

The fall velocity of pellets produced at different times of the year showed limited variation. Pellets produced in April, October and January ($y = 0.11 + 25.2241x$, $r^2 =$ 0.76, P = <0.001; $y = 0.12 + 23.8692x$, $r^2 = 0.86$, P = <0.001; $y = 0.21 + 19.4584x$, $r^2 =$ 0.78, P = <0.001, respectively) had tightly clumped regression lines, while the regression line of the pellets produced in July was slightly lower then the others ($y =$ $0.11 + 19.3759x$, $r^2 = 0.73$, P = <0.001) (Figure 4.3). These data were log 10 transformed to provide a normal distribution and were tested using the Kolmogorov-Smirnov test (P = 0.068). ANCOVA showed highly significant differences between the fall velocities of pellets produced from stream water collected at different times of the year (Table 4.5). However Tukey's *post-hoc* test revealed that the only significant differences were between the pellets produced in July 2004 and January 2005 (Table 4.6).

Table 4.5; Results of ANCOVA comparing the fall velocity of pellets produced from larvae at different seasons.

Source	D.F.	Seq SS	Adj SS	Adj MS	F	P
Volume	1	3.64787	3.16587	3.16587	242.02	0.000
Date	3	0.17622	0.17622	0.05874	4.49	0.000
Error	115	1.50433	1.50433	0.01308		
Total	119	5.32842				

Figure 4.3; Regression lines of the fall velocity of pellets produced by larvae at different times of the year relative to the pellet volume.

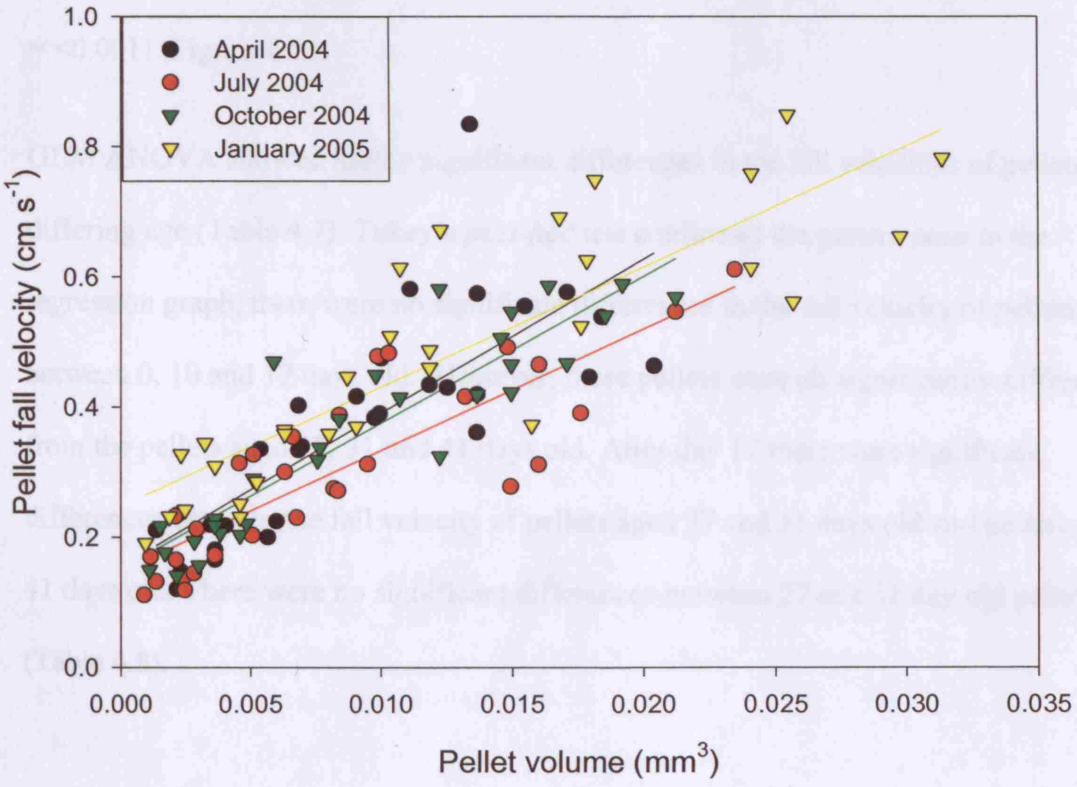


Table 4.6; Results of *post-hoc* Tukey test showing significant differences between the fall velocity of pellets produced from larvae at different seasons. Asterisks represent P values, * P = <0.05, ** P = <0.01, *** P = < 0.001.

Jul-04			
Oct-04			
Jan-05		**	
	Apr-04	Jul-04	Oct-04

4.3.4 Effects of conditioning on the transport of faecal pellets

There was a clear pattern of decreasing fall velocity with increasing age. Pellets that were 0, 10 and 17 days old had closely grouped regression lines ($y = 0.11 + 14.9384x$, $r^2 = 0.69$, $P = <0.001$; $y = 0.12 + 13.7413x$, $r^2 = 0.61$, $P = <0.001$; $y = 0.12 + 14.5056x$, $r^2 = 0.45$, $P = <0.001$, respectively), as the pellets increased in age after 17 days their fall velocity decreases accordingly (27 days $y = 0.08 + 10.4227x$, $r^2 = 0.79$, $P = <0.001$; 31 days $y = 0.05 + 9.8977x$, $r^2 = 0.54$, $P = <0.001$; 41 days $y = 0.04 + 3.4024x$, $r^2 = 0.56$, $P = <0.001$) (Figure 4.4).

GLM ANOVA showed highly significant differences in the fall velocities of pellets of differing age (Table 4.7). Tukey's *post-hoc* test confirmed the pattern seen in the regression graph, there were no significant differences in the fall velocity of pellets between 0, 10 and 17 days old. However, these pellets were all significantly different from the pellets aged 27, 31 and 41 days old. After day 17 there were significant differences between the fall velocity of pellets aged 27 and 31 days old and pellets aged 41 days old. There were no significant differences between 27 and 31 day old pellets (Table 4.8).

Figure 4.4; Regression lines of the fall velocity of pellets of progressively older age relative to the pellet volume.

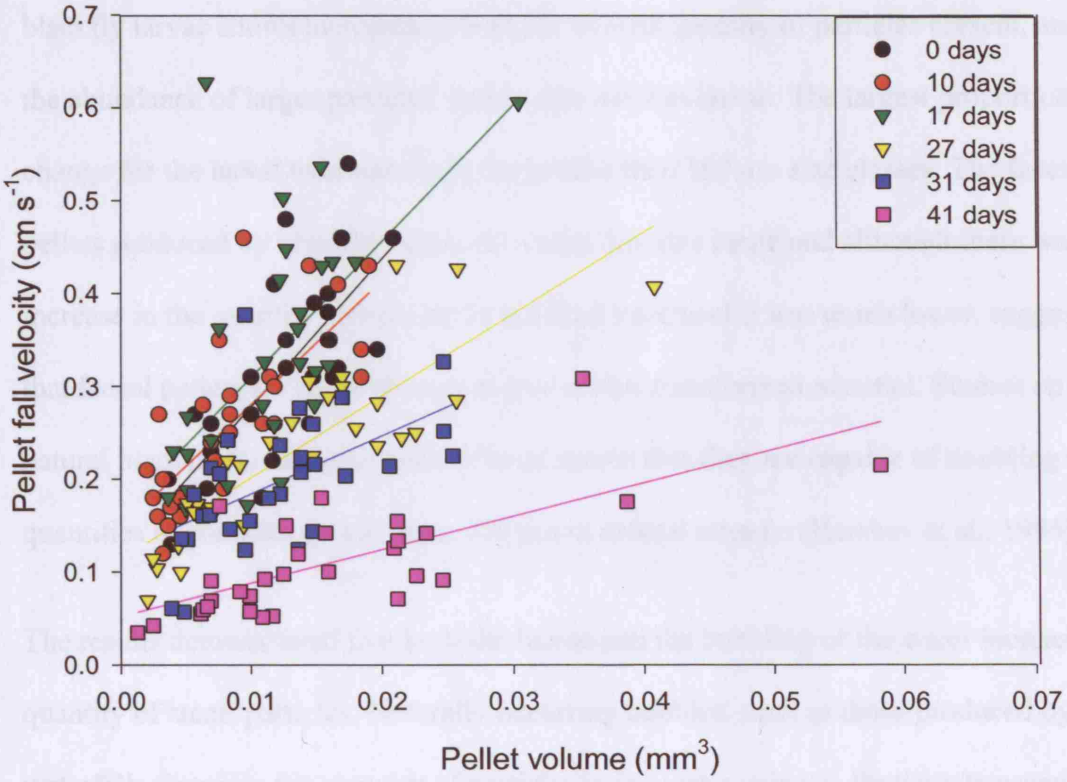


Table 4.7; Results of ANCOVA comparing the fall velocity of pellets of different ages.

Source	D.F.	Seq SS	Adj SS	Adj MS	F	P
Volume	1	0.29799	0.60519	0.60519	110.44	0.000
Age	5	1.25864	1.25864	0.25173	45.94	0.000
Error	173	0.94799	0.94799	0.00548		
Total	179	2.50462				

Table 4.8; Results of *post-hoc* Tukey test showing significant differences between the fall velocity of pellets produced from larvae at different seasons. Asterisks represent P values, * P = <0.05, ** P = <0.01, *** P = < 0.001.

10					
17					
27	***	***	***		
31	***	***	***		
41	***	***	***	***	***
	0	10	17	27	31

4.4 Discussion

Blackfly larvae impact on the size of particles in the water column. Water colonised by blackfly larvae shows increases in both the overall quantity of particles present, and in the abundance of larger particles, than water without larvae. The largest proportional change for the larval treatment is in the greater then 100 μm size classes. The faecal pellets produced by blackfly larvae fit within this size range and although there was an increase in the quantity present in the bubbled treatment it was much lower, suggesting that faecal pellets are the dominant source of this transformed material. Studies on dense natural blackfly larvae aggregations have shown that they are capable of doubling the quantities of particles greater then 100 μm in natural streams (Hershey et al., 1996).

The results demonstrated that both the larvae and the bubbling of the water increase the quantity of small particles. Naturally occurring bubbles, such as those produced by waterfalls, increase the quantity of particles in the water column. Particulate organic carbon increased by 1.2 mg C l^{-1}, while dissolved organic carbon decreased by 0.1 mg C l^{-1} below a waterfall compared to upstream of the waterfall (Petersen, 1986). This study also found an increase in the abundance of particles between 2 – 20 μm, a pattern reflected in the current study.

The larval treatment showed an increase in particles in the size class 1.2 – 25 μm above that produced by bubbling. This increased formation of particles is unlikely to be a result of increased turbulence due to larval attachment to the bucket walls as visual inspection indicated that there was no significant additional turbulence associated with the larvae. Larval aggregations killed by Bacillus Thuringiensis (*Bti)* application led to a decrease in the quantities of 0.7 – 10 μm particles downstream of the aggregation compared to pre *Bti* application, although the differences were not significant (P = <0.1)

(Hershey et al., 1996). Killing blackfly larvae using *Bti* leaves the larvae attached to the substratum, causing minimal variation in turbulence, therefore the increases in particles must be a result of larval activity, most likely feeding. The current study used blackfly larvae from a population with individuals of varied sizes, whereas the size distribution of the aggregation studied by Hershey is unknown and so the high quantities of small particles found in the present study may be a result of larger numbers of small larvae.

The quality of the stream seston and therefore the diet of the blackfly larvae has a significant potential impact on the transport of pellets subsequently produced. The higher density of mineral particles, compared to organic particles, is translated into a higher fall velocity per unit volume. The fall velocity of pellets produced by larvae fed on a natural chalk stream diet were not significantly different from larvae fed exclusively, or entirely, on an organic diet. The distinctive hydrology of chalk streams results in very little wash in of mineral particles from the surrounding catchment as much of the flow will be dominated by base flow from the chalk aquifer. Coupled with high levels of autocthonous primary production it is expected that chalk streams will have a suspended load dominated by organic particles.

Malmqvist et al. (2001) compared the fall velocity of pellets produced by larvae from a lake outlet stream (seston with high levels of organic material) with those produced from a large snowmelt river (seston with high levels of mineral particles). They found that pellets produced from the snowmelt river had a significantly faster fall velocity ($y = 111x - 0.073$) then those produced in the lake outlet ($y = 7.98x + 0.0655$). Using these regression line slopes and comparing them with the slope for the natural chalk stream water (see above, $y = 25.9281x + 0.07$) a pellet of 0.01 mm^3 will have a fall velocity of 1.04 cm s^{-1} for the snowmelt river, 0.15 cm s^{-1} for the lake outlet and 0.33 cm s^{-1} for the

chalk stream. This again suggests that a heavily organic seston is influencing the distances travelled by the faecal pellets produced in chalk streams.

Seasonal influence on the fall velocity of pellets appears to be limited, significant differences were only found between the winter and the summer samples. As baseflow dominates in the summer, decreasing discharge reduces the ability of streams to transport particles. This will reduce the quantity of denser mineral particles in the suspended load as they are preferentially deposited and lead to a highly organic stream seston as high levels of autocthonous production provide a large source of organic particulate inputs. Faecal pellets produced from this material will have a low sinking rate. During winter, storm events and over bank flooding increases the inputs of mineral particles, while higher discharges initiate bank erosion and the mobilisation of bed sediments. Coupled with seasonally low levels of primary production the stream seston has an increased mineral loading and any pellets subsequently produced will have higher fall velocities compared to summer pellets.

Seasonal variation in pellet transport properties will have minimal impacts on chalk stream sediment dynamics for two reasons. Firstly, Tadnoll Brook is an atypical chalk streams as it drains heathland and so has a stronger run-off influence on its hydrology increasing inputs of catchment derived material during storm events. Therefore, if seasonality only exerts a limited influence on the Tadnoll Brook then its impacts on other chalk streams are likely to be minimal. Secondly, winter population densities of blackfly larvae are an order of magnitude lower in comparison to summer populations (Ladle et al., 1972) therefore production of pellets will follow a similar pattern. In addition the overwintering populations tend to be dominated by larvae with small body

length (Ladle et al., 1977) which produce pellets of small volumes, further reducing their impacts on sediment dynamics.

The decrease in the fall velocity of pellets as they age will impact on particulate transport dynamics in streams. Faecal pellets are found in large deposits on the stream substratum, the numbers present suggest that these have accumulated over time and there will thus be pellets of different ages in the deposits. As older pellets have a reduced density in relation to younger pellets then it will be expected that a lower shear stress will be required to initiate particle transport. Thus deposited pellets as they age and decrease in density may cross a critical threshold in relation to ambient shear stress values initiating uplift of the pellets into the water column and further transport downstream. Alternatively, increases in shear stress will have a winnowing effect on pellets by preferentially entraining older pellets into the water column and transporting them downstream.

Models on entrainment of mineral particles from the stream bed consider the principal control on the shear stress needed to induce particle transport to be particle size (Knighton, 1998), presumably variations in mineral particle density are too small to exert a strong influence on the critical shear stress for entrainment. However, with aggregates containing a wide variety of constituent particles and subject to modification over time then density will be expected to become an increasingly important factor in transport initiation. Laboratory experiments on the shear stress thresholds required to erode faecal pellets from the mussel *Perna canaliculus* found that pellets produced on an algal diet were less dense then those produced from both a natural and silt diet and subsequently required a lower shear stress to initiate transport (Giles & Pilditch, 2004). Additionally, factors initiating mass entrainment of deposited pellets occur in streams

such as weed cutting, increases in discharge and biotic disturbance. As pellets show a decrease in the depositional velocity with increasing age then the older pellets will be dispersed further downstream before being redeposited onto the stream substratum in comparison to younger pellets.

Dispersion of blackfly faecal pellets will be controlled by a variety of factors. Initially, by the relative contributions of mineral and organic matter to the stream seston; this directly influences larval diet, the fall velocity of pellets subsequently produced and the degree of longitudinal dispersal. Qualitative variation of the stream seston and its impacts on blackfly mediated sediment dynamics will be minimal. In chalk streams peak larval populations coincide with stable flow regimes and larvae will not experience large variations in the proportions of organic to mineral components of the seston. Between river differences in the transport of blackfly pellets can be high, particularly between rivers exhibiting contrasting hydrological regimes. The origins of the flow and its annual variability change the nature of particles in the stream seston and so cause variation in pellet dispersal. The age of pellets exerts a strong influence on fine sediment dynamics in chalk streams, large fresh pellets sediment rapidly to the substratum. As they age their density will decrease as a result of increased porosity during conditioning leading to preferential dispersal and downstream transport of older pellets.

The transformation of fine suspended particles into much larger faecal pellets by blackfly leads to the transfer of this material to the substratum as larger particles are subject to stronger gravitational forces than smaller particles and sink more rapidly. Blackfly larvae therefore reduce the spiralling distance of particles (distance travelled as a particle is captured, retained and released) that they intercept (Wotton et al., 1996). As

the distance particles travel downstream is shorter when large populations of blackfly larvae are present, then there is increased efficiency in the utilisation of particles within the stream ecosystem (Wallace et al., 1977).

Streams with high population densities of blackfly larvae demonstrate a significant degree of detritus retention. Deposits of stream detritus have an associated microbial community attached to the detritus particles, this community consists of both bacteria and fungi that use the detritus as a substratum and a source of dissolved organic matter (Meyer, 1994). These microbes are consumed by protozoa, meiofauna and macroinvertebrates thus providing a link with higher trophic levels (Hall & Meyer, 1998). Bacteria, associated with detritus deposits trapped by woody debris, can contribute over 90% of carbon required for the growth of mayfly larvae (Edwards & Meyer, 1990). The feeding activity of blackfly larvae impacts on the stream ecosystem beyond that of simple resource utilisation for food. The transformation of the material and the influence this has on particulate dynamics has important implications for fine sediment and nutrient dynamics within these systems.

5 Test of factors influencing the transport of particles in chalk streams

5.1 Introduction

Rivers are conceptualised as longitudinal systems with strong linkages between upstream and downstream reaches as the continual, downstream flow of water means that adjacent reaches are heavily reliant on processes occurring upstream. The transport of organic material has important implications for the transport of nutrients and energy to downstream reaches (Vannote et al., 1980). A simplified view of organic matter processing in streams sees the continual processing of material into ever-smaller particles. Thus Coarse Particulate Organic Matter (>1 mm) enters the stream and is trapped in the channel, where it is subject to fragmentation through abrasion and invertebrate consumption to become Fine Particulate Organic Matter (FPOM) (<1mm - >0.45mm). Concurrent with this decrease in particle size is an increase in the distance that particles are transported (Allan, 1995).

The importance of transport processes to stream ecosystem processes has led to attempts to quantify these processes using analogue or surrogate particles. These studies invariably utilise some form of release and retrieval methodology which enables the degree of retention of the CPOM analogues in the stream environment to be determined. This allows factors that influence CPOM transport to be tested through manipulations of the particles and the stream environments (Ehrman & Lamberti, 1992; Diez et al., 2000; Brookshire & Dwire, 2003; Horvath, 2004).

More recently the quantification of transport processes for FPOM have been attempted. This has previously been hampered by the difficulty in recapturing and identifying these very fine particles in comparison to CPOM analogue particles which are more readily

identifiable. Advances in the labelling of fine organic particles has overcome some of the inherent difficulties in identifying recaptured particles and has allowed the methodologies used in CPOM studies to be adapted for FPOM. A variety of FPOM analogues have been utilised each with different sizes and therefore transport capabilities. These include stained corn pollen, 87 μm (Miller & Georgian, 1992); labelled *Lycopodium clavatum* spores, 42 μm (Reynolds et al., 1990; Wanner & Pusch, 2000); fluorescently labelled yeast, 6 μm (Paul & Hall, 2002); fluorescently labelled bacteria 2 μm (Hall et al., 1996) and radiolabeled natural FPOM (Cushing et al., 1993; Minshall et al., 2000). Most studies have focused on using these analogues to estimate the distance that FPOM travels in the natural environment (Miller & Georgian, 1992; Cushing et al., 1993; Wanner & Pusch, 2000). Whilst others have used these techniques to investigate the trapping efficiency of different stream features (Wanner & Pusch, 2001), the influence of hydrological features of streams (Minshall et al., 2000; Paul & Hall, 2002), or the capture of FPOM by invertebrates (Hall et al., 1996). However few have used FPOM analogue particles in association with stream environment manipulations as has been done with CPOM analogues.

The high transport capabilities of FPOM in streams means that the retention of this material is determined by both flow patterns and the presence of structures that act to trap this material within the stream (Allan, 1995). It was the aim of this study to use corn pollen as a FPOM analogue and to manipulate the stream environment to determine the influence of different factors on the transport of organic particles in chalk streams. Corn pollen (Polysciences, Eppelheim, Germany) was selected as the most suitable analogue particle for this study for a number of reasons. Firstly it is in the size range of blackfly faecal pellets, albeit towards smaller blackfly pellets. Therefore the results will be indicative of how at least a proportion of the blackfly faecal pellets are

transported in these systems. Secondly, the acquisition and staining of corn pollen presented no logistical problems, and finally there were no environmental concerns with the release of this material into the environment.

5.2 The role of macrophytes in the transport of particles

Macrophytes are a key component of the chalk stream ecosystem and abundant macrophyte communities are one of the most noticeable features of these rivers (English Nature & Environment Agency, 1999). These systems support high macrophyte abundance, with macrophytes covering up to 76 % of the channel surface in the Frome / Piddle catchment (Wharton et al., 2006). Indeed macrophyte biomass can reach such high levels that traditional chalk stream management practises involve the periodic cutting and removal of the macrophytes to prevent flooding and improve fisheries (Dawson, 1976b; Dawson, 1978; Hearne & Armitage, 1993; Wright et al., 2003).

Within macrophyte stands there is typically a steep reduction in the flow velocity in comparison to the ambient flow conditions (Gregg & Rose, 1982; Sand-Jensen & Mebus, 1996; Madsen et al., 2001; Clarke, 2002). The key determinant of the ability of a macrophyte species to influence flow is the physical nature of the tissues within the water column. Species with an upright, open growth habit and streamlined leaves provide little resistance to flow and have only a limited impact on flow velocity. In contrast, species which develop dense, closed stands create steep velocity gradients at the plant-water interface (Sand-Jensen & Pederson, 1999).

The reduction in flow velocities promotes a depositional environment, encouraging the trapping and accumulation of fine sediments within stands (Gregg & Rose, 1982; Sand-Jensen et al., 1989; Wood & Armitage, 1999; Madsen et al., 2001; Clarke, 2002). Studies on a low-gradient stream in the USA found that, in the presence of *Sparganium americanum*, the quantity of organic matter deposited on the stream substratum was greatly elevated after macrophyte removal. Additionally, the reach with the *Sparganium*

present showed increased retention of both FPOM and CPOM (Koetsier & McArthur, 2000). Fine sediments trapped within macrophyte stands accumulate over the course of the growing season. The structure of the macrophytes shields these deposits from disturbance events thereby increasing the retentive capabilities of the reach. The fine sediments are only re-mobilised when higher discharges during winter erode the macrophytes from the river channel, exposing the sediments to the flow and initiating resuspension (Sand-Jensen & Mebus, 1996).

An understanding of the role that macrophytes play in the dynamics of fine sediments is of increasing interest as fine sediments are cited as a major cause of environmental degradation in many chalk streams (Walling & Amos, 1999). Fine sediments can have wide ranging impacts on stream ecosystems. Primary producers suffer through increased turbidity, reducing the available light for photosynthesis. High levels of deposited fine sediments can lead to a reduction in the breeding success of salmonids as the infilling of interstitial gaps in a gravel bed reduces the availability of oxygen for egg development. In addition, many contaminants are tightly bound to fine sediments and their fate is tightly linked to that of the sediment (Wood & Armitage, 1997).

The presence of macrophytes at very high abundances, coupled with the known association of macrophytes with fine sediment has necessitated process-driven research into their interaction. Although the factors that influence fine sediments are diverse, in chalk streams the macrophyte component of these dynamics is expected to be of great importance. The distinctive seasonal nature of macrophyte growth, coupled with their discrete growth forms results in macrophytes exerting a strong temporal and spatial element on fine sediment dynamics.

It is expected that corn pollen released into streams with high *Ranunculus* abundance will have increased retention within the reach as the *Ranunculus* traps and stores the corn pollen, compared to corn pollen released into reaches with low *Ranunculus* abundance.

5.2.1 Methods

5.2.1.1 Corn pollen preparation

Previous studies using corn pollen as a seston surrogate have stained the pollen either prior to release (Georgian et al., 2003) or after collection as part of the counting process (Miller & Georgian, 1992). To avoid possible contamination from cultivated corn and to make the staining process easier it was decided to bulk stain the pollen pre-release, this has been shown to have no affect on pollen characteristics (Georgian et al., 2003). Corn pollen was stained using basic Fuchsin, using a solution consisting of 5 ml of glycerol, 10 ml of ethanol, 15 ml distilled water to which 0.5 g of basic Fuchsin was added (Zangerl et al., 2001). The corn pollen was poured into the stain, shaken and left for several minutes. Excess stain was removed by rinsing with distilled water. The pollen was stored in 60 ml wide neck bottles and a small quantity of water was added to enable the pollen to be easily rinsed out in the field.

5.2.1.2 Trial release

A conservative tracers, NaCl, was used to determine the discharge and mean flow velocity of the stream (Miller & Georgian, 1992). A trial of the techniques needed for the releases of the corn pollen and NaCl was undertaken at the Bere Stream in early 2004. The aims were to determine if the corn pollen and NaCl could be successfully quantified as it passed downstream sites. The corn pollen was mixed with 6 L of stream water and released into the stream using a siphon tube attached to a 60 cm aquarium

spray bar. Corn pollen was recovered at all sites downstream of the release points up to 100 m. However, there were significant differences between the peaks found at the various sites, indicating that the sampling intervals were too widely spaced apart.

The NaCl solution was released using a steady head release tank. This consisted of two tanks; a bottom tank which releases the solution into the stream and an upper tank, which maintains a constant head of water in the lower tank to ensure a steady rate of release of salt solution (J. Hope, pers. comm.). The conductivity values at 30 and 100 m downstream of the sampling sites showed no significant differences above the background levels. The release of NaCl solution using the steady head release tank was unsuitable as it was impossible to raise conductivity levels substantially. A subsequent 'gulp' release of NaCl was successful in raising the background conductivity by a significant degree and this technique was considered the most suitable to release the NaCl.

5.2.1.3 August 2004

Measurements of corn pollen transport were undertaken at Bere Stream, immediately downstream of Snatford Bridge. This reach was selected as it had a small pool just downstream of the release point that would aid the mixing of the corn pollen and NaCl with the stream water. Downstream of the pool the channel was divided into two by a heavily vegetated mid-channel bar. One of the side channels had no *Ranunculus* growth and consisted of a bare cobble substratum, the other had substantial *Ranunculus* growth in the channel. To see differences in particle transport and the hydrological characteristics of the reaches sampling sites were set up at the upstream and downstream ends of each channel. Each sampling site required one individual to collect the samples.

The channel width was measured every meter to obtain mean stream width and the areal extent of macrophyte growth mapped to provide the percentage cover of macrophytes. An electromagnetic flow meter was used to determine stream velocity at 0.5m intervals along a transect crossing the channel. The mean velocity for the channel was multiplied by stream cross-sectional area to determine stream discharge. To determine the hydrological characteristics 10 kg of NaCl was fully dissolved into 50 L of stream water in a black plastic rubbish bin and 3.5 g of corn pollen was added. The tracers were released upstream of the pool as a gulp release, at the moment the tracer was released a whistle was blown and a stopwatch started. Samples were taken after the first ten seconds and at ten second intervals up to 3 minutes, then samples were collected every 20 seconds up to 5 minutes post-release. Samples were collected in pre-marked, re-sealable plastic bags, with an approximate capacity of 1.1 L. To minimise leakages all samples were double bagged and placed in a single layer in plastic trays to be transported back to the laboratory for analysis.

In the laboratory, samples were placed on a laboratory bench and allowed to equilibrate with ambient laboratory temperature as conductivity of NaCl solution is related to the temperature of the solution (Church, 1974). A series of seven NaCl standards, varying from 13 mg L^{-1} to 1015 mg L^{-1}, were used to calibrate the conductivity meter. These were left alongside the field samples to reach ambient laboratory temperature. All conductivity readings were taken with a HANNA Instruments Conductivity Meter, Model HI8733. Prior to measuring the conductivity of the samples, measurements were made of the standards. Readings were made by dipping the meter's probe into the sample and stirring gently until a steady value was obtained. Between measurements the probe of the meter was dipped into distilled water and then rinsed with distilled water from a wash bottle to reduce contamination of the samples.

To count the number of pollen grains collected, each sample was poured into a 1 L graduated glass measuring cylinder and the volume of water in the bag recorded. The sample was then poured through a 25 μm Monyl net and rinsed under a tap to remove any small particles after which it was transferred to a 55 mm diameter plastic Perspex Petri dish. The base of the dish was marked with a grid to ensure that no pollen grains were counted and the entire dish scanned for corn pollen under a dissecting microscope. The pollen was easily identified as the bright red stain on the surface of the pollen grains was readily distinguished from other stream material. The total number of particles in each sample was counted and values changed to number of pollen grains per litre.

5.2.1.4 June 2005

To investigate the impact of *Ranunculus* on the transport of corn pollen on a larger scale, releases were conducted on whole river reaches. The first release was on a reach immediately downstream of the August 2004 sampling sites, this section of the Bere Stream flows through a field and has extensive macrophyte growth due to the high light levels (Figure 5.1). Immediately downstream of this reach the river flows through woodland, the high degree of shading within the woods prevents the growth of macrophytes (Figure 5.2) and this reach was used to contrast the transport of pollen through a *Ranunculus*-free reach.

The physical characteristics of the streams were determined by mapping a series of nine transects through each of the reaches. The *Ranunculus* reach was 44 m long and at each transect the macrophyte cover across the stream was mapped and depth measurements made at 1 m intervals for five of the transects. The downstream reach contained no macrophyte growth and so only stream widths and depths were recorded.

Figure 5.1; Reach at Bere Stream containing abundant macrophyte growth, June 2005. This reach flows into a wood which can be seen in the picture.

To save time processing the samples and to provide a clearer picture of the required sampling intervals for the corn pollen, the passage of the NaCl tracer was measured *in situ* using conductivity meters. At both of the reaches 12 kg of NaCl was dissolved into 60 L of stream water in a plastic rubbish bin and released as a gulp 15 m upstream of the reach to allow mixing of the tracer. Conductivity readings were taken at the upstream and downstream end of each sample reach. Readings were taken every 15 s for 12 mins after releases of the pollen grains into the water. After the conductivity readings had been made the meters were calibrated with NaCl standards. These were kept in sealed, 200 ml Schott Duran reagent bottle that had been placed in the stream prior to the release to equilibrate with the stream temperature.

Figure 5.2; Reach at Bere Stream after entering the wood. In contrast to the reach in figure 5.1 the low light levels prevents the growth of macrophytes.

The corn pollen was mixed with 3 L stream water in a plastic bucket and released at the same point as the NaCl. Samples were collected at both the upstream and downstream ends of the sampling points using the technique described above for August 2004 (see section 5.2.1.3). The sampling interval for the *Ranunculus*-free reach was every 10 seconds up to 3 minutes, then every 29 seconds to 6 minutes and finally every 30 seconds up to 12 minutes after the release of the corn pollen. Sampling stopped at the upstream site after 6 minutes as the NaCl tracers indicated that the released material would have completely passed the upstream site by this time. For the *Ranunculus* reach

the NaCl tracer indicated that a longer sampling interval was needed due to slower passage of the material through the reach. Samples started at every 10 seconds to 4 minutes, then every 20 seconds to 6 minutes and finally every 30 seconds to 14 minutes. The post-capture treatment and processing of the samples followed the method used in August 2004.

5.2.1.5 July 2005

The second sets of releases in July 2005 were completed in the *Ranunculus* reach used in June 2005, allowing seasonal differences in the transport of particles through *Ranunculus* to be recorded (Figure 5.3). After the release of the tracers through the *Ranunculus* reach all the macrophytes were removed from the reach (Figure 5.4). The macrophytes were removed by pulling them up from the substratum and transferring them to the stream bank. The removal of the macrophytes allowed comparison of particle transport through the reach to be made both in the presence of the *Ranunculus* and without *Ranunculus* and allows the effects of *Ranunculus* on particle transport to be determined. The release post removal of the *Ranunculus* allowed the passage of the corn pollen through two different *Ranunculus*-free reaches to be compared. The reach under investigation was 40 m long with a 15 m mixing zone upstream of the top sampling site. The methods for the release of the NaCl and corn pollen were the same as those used in the June 2005 release (see section 5.2.1.3). After the first release of the corn pollen through the *Ranunculus* reach all the in-channel vegetation was removed and the system allowed to equilibrate for 24 hours before the second release.

Figure 5.3; Reach at Bere Stream, July 2005. This is the same reach as that used in June 2005, see figure 5.1.

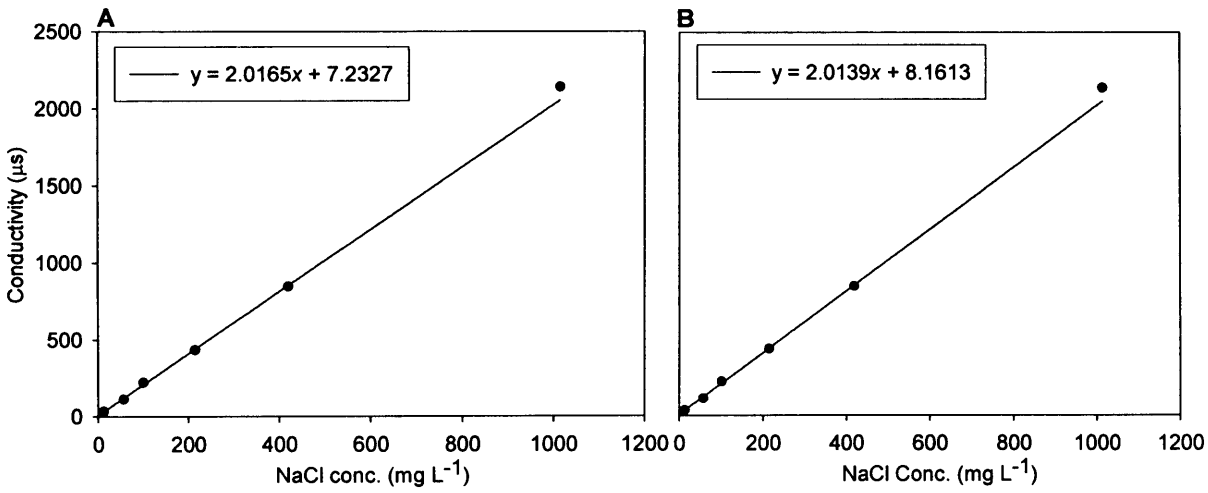
Figure 5.4; Bere Stream reach following the removal of the macrophytes from the channel.

5.2.2 Results

5.2.2.1 August 2004

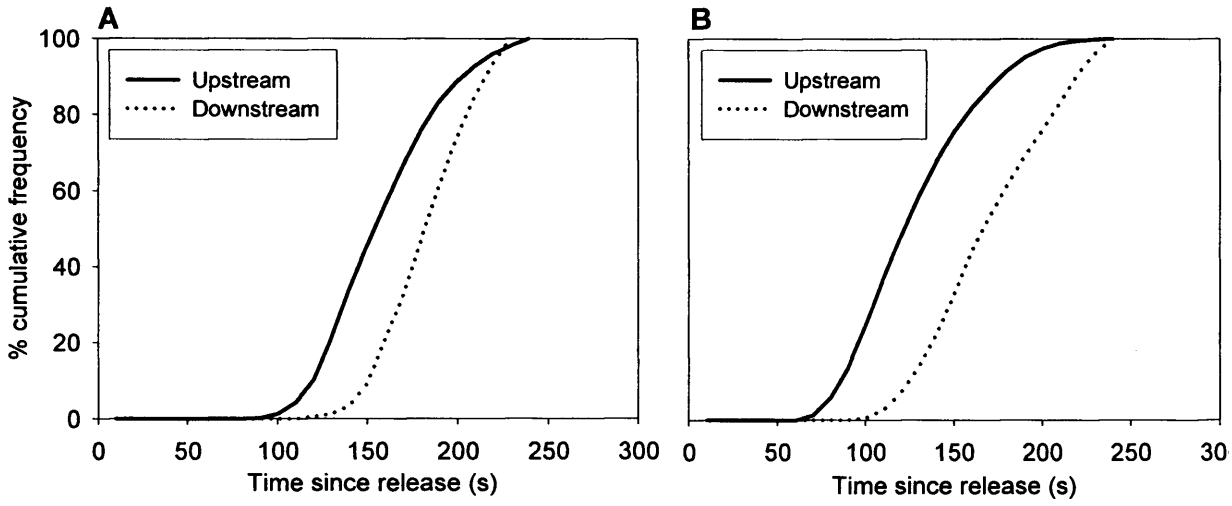
The *Ranunculus* and *Ranunculus*-free reach had distinct differences in their hydraulic and morphological characteristics. The *Ranunculus* reach had a narrower mean stream width, 0.9 m, and higher mean water depth, 0.4 m and lower discharge, 0.09 $m^3 s^{-1}$, then the *Ranunculus*-free reach, 1.8 m, 0.17 m and 0.16 $m^3 s^{-1}$. The *Ranunculus* reach had 47 % of the channel surface covered by the *Ranunculus*, while only 8 % of the *Ranunculus*-free reach was covered with *Ranunculus* (Table 5.1).

Figure 5.5; NaCl calibration curves produced from the standards. Measurements taken on A) 19/08/04 and B) 20/08/04.



Calibration curves were constructed using NaCl standards of 13, 57, 101, 215, 420 and 1015 mg l^{-1} (Figure 5.5). Conductivity readings were taken over two days, the 19th & 20th August 2004, therefore readings were taken of the standards each day to control for differences in room temperature that would impact on the readings. The standard readings were used to produce the line equation $y = mx + b$, this was manipulated to $x = (y - b) / m$, so that conductivity readings could be used to calculate NaCl concentration.

Figure 5.6; Percentage cumulative frequency plots of NaCl as it passes through a reach with A) *Ranunculus* present and B) a reach with no *Ranunculus* growth.

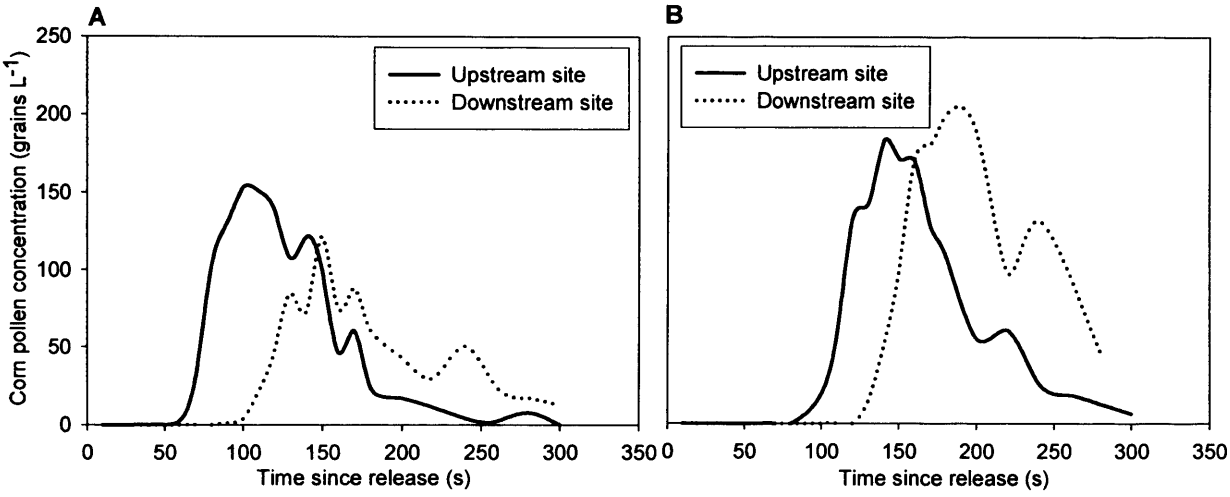


The conductivity measurements were used to calculate the total NaCl passing each of the sampling points and percentage cumulative frequency curves constructed (Figure 5.6). The Nominal Transport Time (NTT) for the reach was calculated as the time taken for 50 % of the NaCl to pass between the upstream and downstream sampling sites. The distance between the two sampling sites was then divided by the NTT to give the mean velocity of the water flowing through the reach (D'Angelo et al., 1991). The *Ranunculus* reach had a higher mean velocity (0.38 m s^{-1}) then the reach without *Ranunculus* (0.32 m s^{-1}) (Table 5.1).

At each of the sampling sites, time-concentration curves were constructed to illustrate the passage of grains through the reaches (Figures 5.7). Integration of the area under the curves provides the total number of grains passing each point per litre of stream water. These data were used to compare differences in corn pollen concentration between two parallel reaches due to variation in discharge over the reach, instead of total corn pollen grains in transport through the reach. The difference in the number of grains passing the upstream sampling site from those passing the downstream site allows the retention of

corn pollen within the sample reach to be calculated. The reach containing *Ranunculus* had little retention of grains with only 0.4% retained within the reach. The reach without the *Ranunculus* had substantially increased retention of grains with 22.3 % trapped within the reach (Table 5.1).

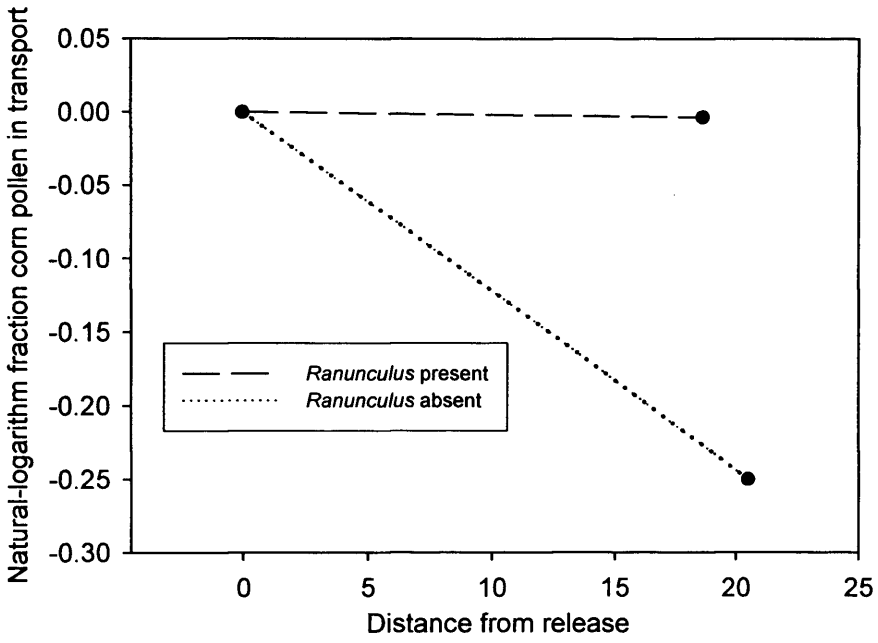
Figure 5.7; Concentration-time plot of corn pollen as it passes through, A) a reach with no *Ranunculus* present, B) a reach with extensive *Ranunculus* growth, August 2004.



The slope of the line, when the natural logarithm of the fraction of grains in transport is plotted against distance from the release site, represents the longitudinal loss rate of particles per meter of stream length (K_L) from the water column (Figure 5.8). The loss rate of particles from the *Ranunculus* reach (slope = -0.0002) is considerably less then that from the *Ranunculus*-free reach (slope = -0.0109). The inverse of K_L is proportional to the median transport distance (S_W) of the particles (Georgian et al., 2003), these were 4651 m for the *Ranunculus* reach and 81.3 m for the *Ranunculus*-free reach. The V_{dep} allows comparisons between systems as it controls for the dual effects of stream depth and channel velocity on the transport of the particle and is calculated by multiplying the K_L by mean water depth and mean velocity and is presented as mm s^{-1} (Minshall et al.,

2000). The *Ranunculus* reach had a V_{dep} of 0.327, this increased to 0.669 in the *Ranunculus*-free reach.

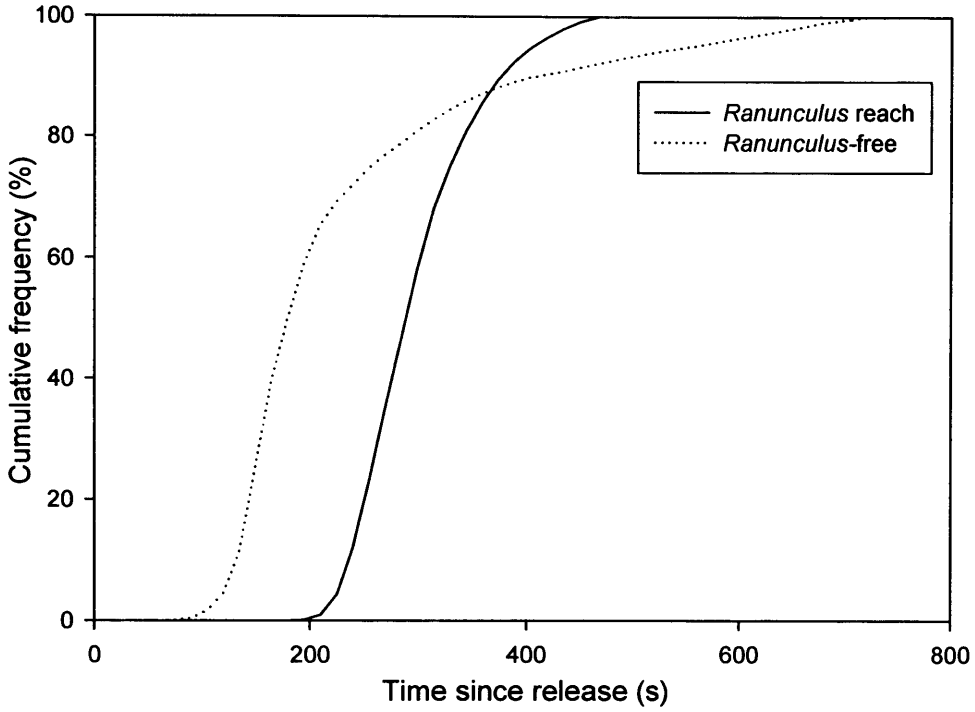
Figure 5.8; The natural-logarithm of the fraction of corn pollen in transport with the distance from release, August 2004. The slope of the line represents K_L.



5.2.2.2 June 2005

The longer adjacent reaches used to examine corn pollen transport at the larger scale had different channel morphologies. The macrophyte reach had very high levels of macrophyte abundance, dominated by *Ranunculus*, *Apium nodiflorum* and *Rorippa nasturtium-aquaticum* and covered a total of 78 % of the reach. The growth of the *Ranunculus* extended up through the entire water column with many of the stems floating on the surface. As the stream entered the heavily-shaded reach all macrophyte growth was inhibited. The *Ranunculus* reach had a mean channel width of 6.27 m compared to 10.96 m for the *Ranunculus*-free reach. The mean depth of the *Ranunculus*-free reach was 0.13 m, the *Ranunculus* reach was substantially deeper at 0.38 m. The discharge for both reaches was 0.337 $m^3 s^{-1}$ (Table 5.1).

Figure 5.9; Cumulative frequency curves for the NaCl tracer release June 2005.



The NaCl standards produced the line equation $y = 1.89x + 18.99$, this was manipulated and the conductivity readings converted to NaCl concentration. The NTT derived from the NaCl release showed that the *Ranunculus* reach had a reduced mean water velocity of 0.204 m s^{-1} as it passed through the reach in comparison to the *Ranunculus*-free reach which was 0.278 m s^{-1} (Figure 5.9). The electromagnetic flow meter readings showed the Thalweg velocity in the *Ranunculus* reach to be similarly reduced (0.43 m s^{-1}) in comparison to that in the *Ranunculus*-free reach (0.647 m s^{-1}) (Table 5.1) (see section 5.2.2.1 above for description of these methods) .

The integration of the area under the time-concentration curves showed the mixing length in the *Ranunculus*-free reach to be too short and the peak passed without being sampled adequately. Therefore the peaks at the downstream sites were used to calculate the total numbers of corn pollen grains exiting the reach and this was compared to the total number of corn pollen grains that was released into the stream originally.

Figure 5.10; Time –concentration curves for corn pollen as it travels through A) *Ranunculus* reach and B) *Ranunculus*-free reach, June 2005.

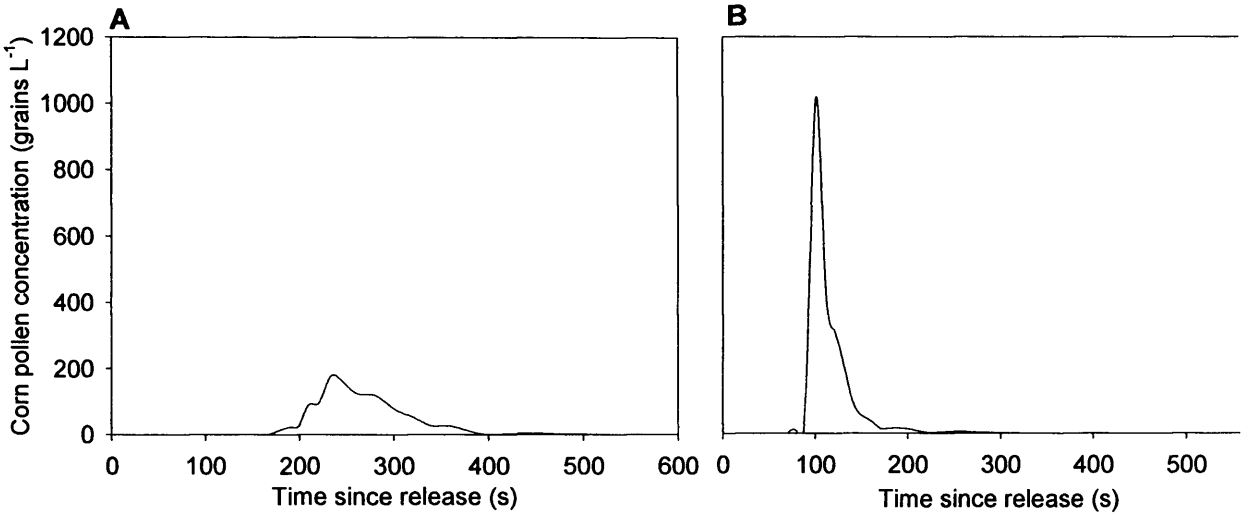
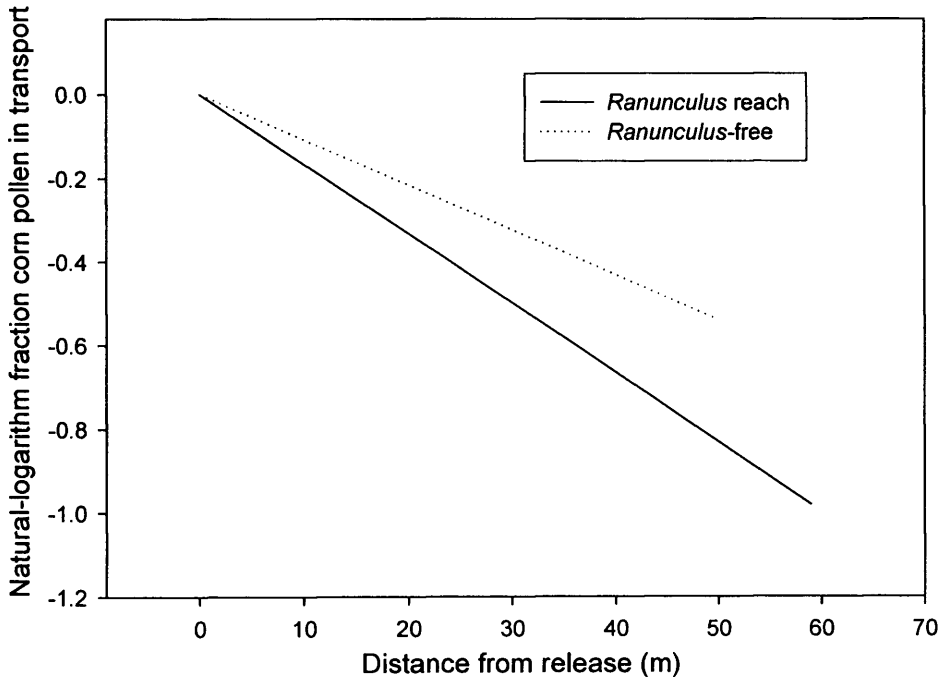


Figure 5.11; The natural log of the fraction of corn pollen in transport against the distance from release June 2005. The slope of the lines are equal to K_L (Table 5.1).



The passage of the corn pollen through the two contrasting reaches showed distinctly differing patterns. The corn pollen in the reach containing abundant *Ranunculus* growth was considerably attenuated with a much lower peak corn pollen concentration of 178

grains L^{-1} than the corn pollen in the *Ranunculus*-free reach, 1013 grains L^{-1} (Figure 5.10). The *Ranunculus* reach retained 62.5 % of the corn pollen while only 41.8 % of the corn pollen was trapped in the *Ranunculus*-free reach (Table 5.1). The loss rate of the corn pollen in the *Ranunculus* reach showed a K_L of -0.017, this was higher then that in the *Ranunculus*-free reach -0.011 (Figure 5.11), the two reaches had S_W values of 58.8 and 90.91 m and V_{dep} values of 1.318 and 0.398 mm s^{-1} respectively.

5.2.2.3 July 2005

By July, the macrophyte abundance at the Bere Stream site had decreased to 63 %. Additionally, the *Ranunculus* had changed its growth form from the floating type seen in June to a more compressed form in July. The *Ranunculus* no longer floated at the surface and the majority of the biomass was contained below the water surface with the stream flow passing over the top surface of the plant. There was considerable growth of *Apium nodiflorum* and *Rorippa nasturtium-aquaticum* into the channel from the stream margin and this acted to further restrict the channel. The modification of the channel vegetation led to changes in the hydrology of the *Ranunculus* reach. The NaCl tracer release (standards produced line $y = 1.83x + 27.64$) showed that in comparison to June the mean velocity of the reach had increased to 0.301 m s^{-1}, similarly the thalweg velocity increased to 0.552 m s^{-1}. After removal of the vegetation mean velocity increased to 0.324 m s^{-1} (Figure 5.12) although thalweg velocity decreased to 0.389 m s^{-1} (Table 5.1).

Figure 5.12; Percentage cumulative frequency curves for the NaCl tracer release July 2005.

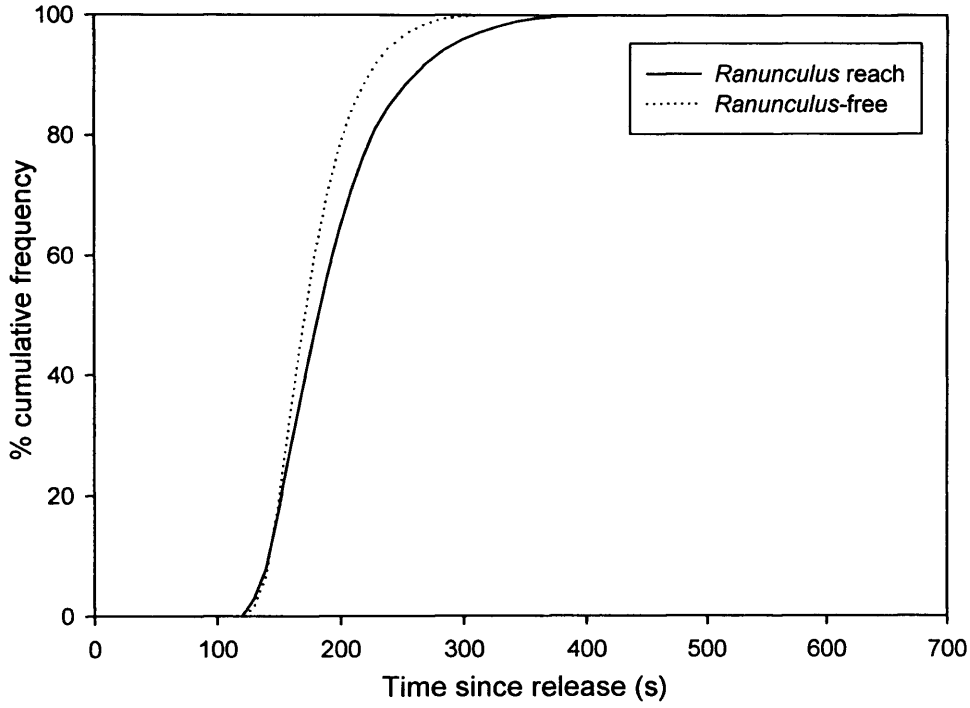
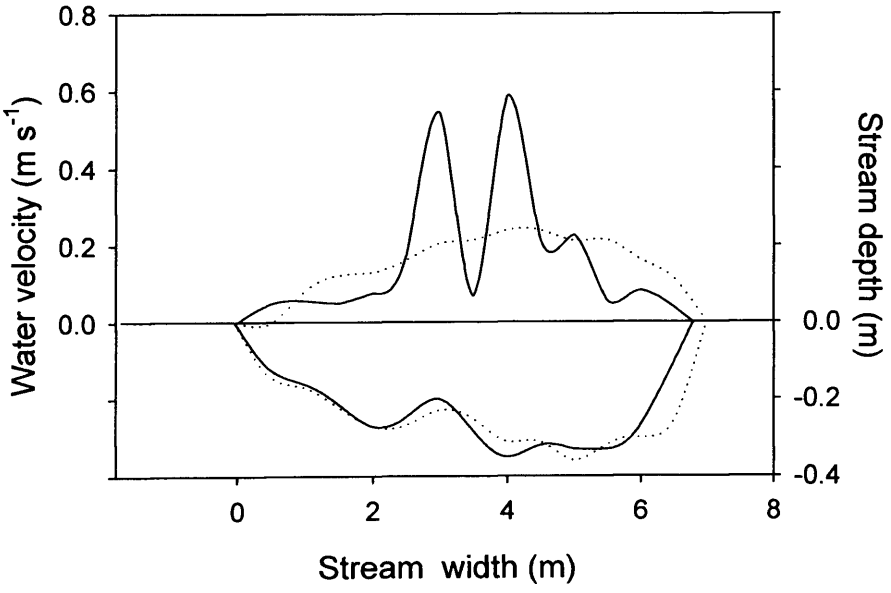


Figure 5.13; Transect measurements across the channel at Bere Stream with and without macrophytes, plots above the y = 0 line represent water velocity and values below y = 0 show stream depth. Solid lines show the depth and velocity profiles in the *Ranunculus* reach while dotted lines show the reach after the removal of all macrophytes.



The removal of the vegetation resulted in some changes to the physical dimensions of the reach, mean water depth decreased from 0.31 to 0.29 m and channel width decreased slightly from 6.36 to 6.28 m. The reach discharge had not varied substantially between June and July, the *Ranunculus* reach had a discharge of 0.363 $m^3 s^{-1}$ and the *Ranunculus* free reach 0.313 $m^3 s^{-1}$ (Table 5.1).

The impact of vegetation removal on the stream characteristics can be seen by comparing transects taken at identical locations before and after the removal of the stream vegetation. The patterns of flow change markedly from a highly heterogeneous flow pattern in the *Ranunculus* reach where areas of relatively high flows, up to 0.6 m s^{-1} alternate with areas of much lower flows, typically less than 0.1 m s^{-1}. In contrast the *Ranunculus*-free reach had a more homogenous flow pattern, the highest velocity recorded was only 0.24 m s^{-1} however the majority of flows recorded were > 0.1 m s^{-1} and therefore were higher than those in the *Ranunculus* reach. The removal of the vegetation had a limited impact on the pattern of sediment deposition within the stream as seen by the changes in the water depth of the channel (Figure 5.13).

The differences seen in the stream hydrology between June and July were mirrored by changes in the transport of the corn pollen through the reach. The time-concentration curves showed a reversal in the patterns of corn pollen transport in comparison with June. The *Ranunculus* reach had a well developed peak of 225 grains L^{-1} at the downstream sampling site (Figure 5.14A), similar to that seen in the *Ranunculus*-free reach in June, although the peak concentration was much lower (Figure 5.10B). The *Ranunculus*-free reach had a similar peak concentration of over 200 grains L^{-1} but the passage of the grains was attenuated in comparison to the *Ranunculus* reach (Figure 5.14B).

Figure 5.14; Time–concentration curves for corn pollen as it travels through A) *Ranunculus* reach and B) *Ranunculus*-free reach, July 2005.

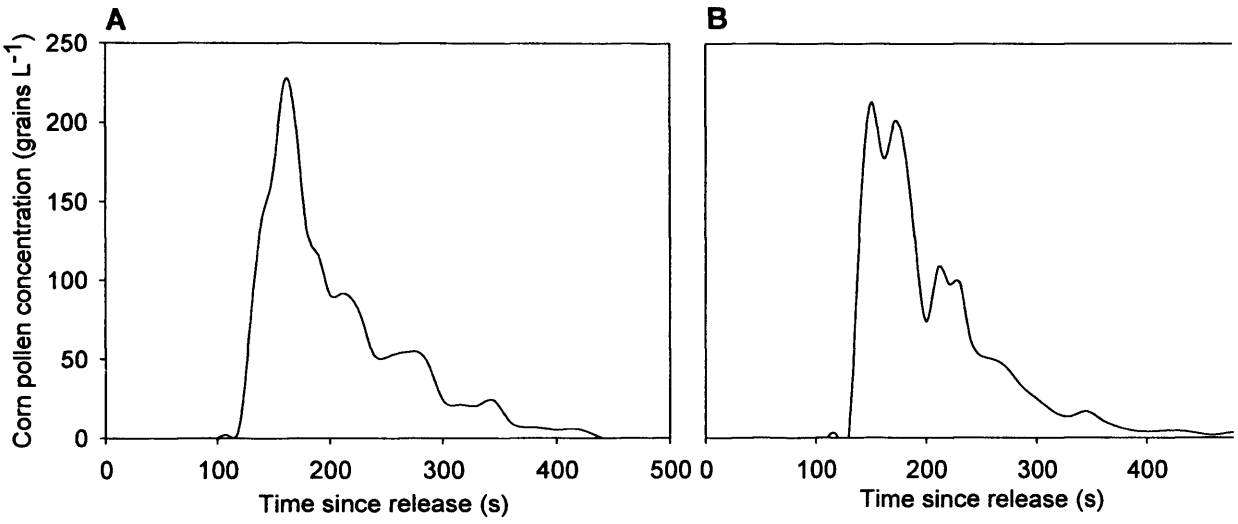
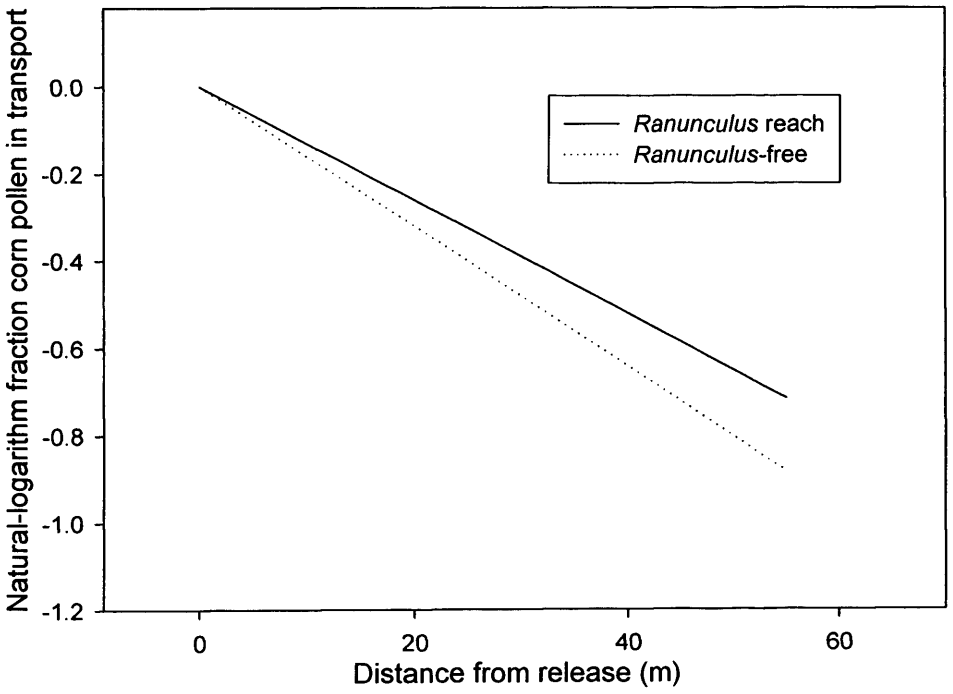


Figure 5.15; The natural log of the fraction of corn pollen in transport against the unmodified distance from release July 2005. The slope of the lines are equal to K_L (Table 5.1).



The *Ranunculus* reach was less efficient at trapping corn pollen than the *Ranunculus* free reach, trapping 51.2% of the corn pollen compared to 58.7 % after the removal of

the macrophytes. This relates to a K_L of -0.013, S_W of 76.9 m and a V_{dep} of 1.21 mm s^{-1} for the *Ranunculus* reach and a K_L of -0.016, S_W of 62.5 m and a V_{dep} of 1.503 mm s^{-1} for the *Ranunculus*-free reach (Figure 5.15) (Table 5.1).

Table 5.1; Physical, hydrological and transport characteristics of the sampling sites.

	August 2004		June 2005		July 2005	
	Ranun. reach	*Ranun.* free	*Ranun.* reach	*Ranun.* free	*Ranun.* reach	*Ranun.* free
Upstr. CP grains	15252*	12251*	14,187,950	14,187,950	14,187,950	14,187,950
Downstr. CP grains	15194*	9514*	5,325,909	8,253,747	6,919,553	5,854,349
CP retention (%)	0.40	22.3	62.5	41.8	51.2	58.7
Macrophyte cover (%)	47	8	78	0	63	0
Mean stream width (m)	0.9	1.8	6.27	10.96	6.36	6.28
Mean Depth (m)	0.4	0.17	0.38	0.13	0.31	0.29
Discharge (m^3 s^{-1})	0.09	0.16	0.337	0.337	0.363	0.313
Reach distance (m)	18.6	20.5	59	50	55	55
Loss rate (K_L)	0.000215	0.0123	0.017	0.011	0.013	0.016
Estimated S_W (m)	4651	81.3	58.8	90.9	76.9	62.5
V_{dep} (mm s^{-1})	0.327	0.669	1.318	0.398	1.21	1.503
NTT (s)	49	64	289	180	183	170
Thalweg velocity	*	*	0.43	0.647	0.552	0.389
Mean Velocity (m s^{-1})	0.38	0.32	0.204	0.278	0.301	0.324

*** Refers to grain concentration L^{-1}, not total numbers passing the sampling site**

5.2.3 Discussion

All of the reaches containing *Ranunculus* growth had increased mean water depth compared to *Ranunculus*-free reaches, a feature commonly seen in macrophyte-dominated rivers (Dawson, 1978; Sand-Jensen et al., 1989; Hearne & Armitage, 1993; Wood & Armitage, 1999). Previous studies on the Bere Stream have demonstrated that reaches with high *Ranunculus* biomass have increased water depth up to four times that of reaches containing no *Ranunculus* growth. This was attributed to an increase in the hydraulic drag of the water as it passed the macrophytes (Dawson, 1976b; Dawson, 1978).

A number of studies have found macrophyte stands to generate a reduction in the water velocity as it enters the stand. This is caused by the high biomass and surface area of the

macrophyte tissue and the dense growth forms of many macrophyte species (Sand-Jensen & Mebus, 1996; Sand-Jensen, 1998). However, in addition to the recorded drops in flow velocity there may be a concurrent increase in the flow of water both over and between stands (Gregg & Rose, 1982; Wood & Armitage, 1999; Sand-Jensen & Mebus, 1996). Sand-Jensen & Mebus (1996) found flow velocities to be 2.6 times greater between stands of *Callitriche cophocarpa* then within. Studies looking at whole river reaches have found macrophytes to cause a decrease in the water velocity (Dawson, 1978; Sand-Jensen et al., 1989; Hearne & Armitage, 1993; Horvath, 2004).

This study found that early in June, when the *Ranunculus* was at high biomass, the mean water velocity decreased compared to the downstream *Ranunculus*-free reach. As the season progressed, *Ranunculus* biomass decreased and marginal vegetation increased leading to a rise in mean water velocity as the channel became increasingly constricted. In August higher mean flow velocities were recorded in the *Ranunculus* reach than in the *Ranunculus*-free reach. Mean water velocity is a measure of the hydraulic retention within the stream (Ehrman & Lamberti, 1992), thus *Ranunculus* generally acts to increase the residence time of the water in the reach until late in the season when the macrophyte community decreases residence time. These mean stream velocity data showed very little correlation with the V_{dep}, indeed in the two larger scale releases in June and July 2005 the two highest V_{dep} values (1.318 and 1.503) were recorded for the highest and lowest mean velocity values, July 2005 = 0.324 m s^{-1} and June 2005 = 0.204 m s^{-1}.

For the releases conducted in 2005 there was a strong correlation between the thalweg velocity and the V_{dep} values. The highest V_{dep} value was recorded in the *Ranunculus*-free reach in July 2005, this coincided with the lowest thalweg velocity of 0.389 m s^{-1}, while

the lowest V_{dep} value was recorded the *Ranunculus*-free reach in June 2005 when mean thalweg velocity was 0.647 m s^{-1}. The lowest value for V_{dep} was recorded for August 2004, however no thalweg velocities were made on this occasion, however it was a heavily constricted channel with a well-developed thalweg and personal observations suggest that this reach had a very high thalweg velocity.

When *Ranunculus* is at peak biomass virtually the whole channel is filled with macrophyte tissue, after the biomass starts to decline then well-formed flow paths develop between the stands and act to increase thalweg velocity and this appears to have a strong effect on the transport of particles through these systems. Studies on *Callitriche cophocarpa*, a plant with a similar growth form to *Ranunculus*, found that at maximum biomass over 79 % of the stream discharge would travel between the stands (Sand-Jensen & Mebus, 1996). Indeed the ability of macrophyte stands to maintain areas of fast-flowing water between stands has led to suggestions that they may have implications in the management of rivers suffering from low flows for the maintenance of habitat diversity (Wood & Armitage, 1999). The chalk stream environment therefore is a patchy mosaic of areas of low velocity and high depositional environments within macrophyte stands interspaced with high flow velocities creating either a low depositional, or even high erosional environment (Madsen et al., 2001).

Studies on the factors initiating the retention of CPOM in streams found that macrophytes increased the quantities of CPOM retained compared to when the macrophytes were removed (Koetsier & McArthur, 2000; Horvath, 2004). Other in-channel obstacles are also important for the retention of CPOM in streams, including cobbles, woody debris and boulders (Edwards & Meyer, 1990; Prochazka et al., 1991; Ehrman & Lamberti, 1992; Diez et al., 2000; Brookshire & Dwire, 2003).

The principal mechanism by which CPOM is retained is typically described as the interception of the particles by channel features. Ehrman & Lambert (1992) found that there was a positive relationship between particle size and the likelihood of the particle being trapped within the system as the more likely it was that the particle will find it hard to bypass a snag. This is a different retention mechanism then that of FPOM which involves the fall velocity of a particle from the water column to the stream substratum (Brookshire & Dwire, 2003) (see chapter 4 for work on fall velocity of particles). In the present study, *Ranunculus* only acted to reduce the retention of corn pollen within the reach compared to the *Ranunculus*-free reach when *Ranunculus* filled the water column thereby actively intercepting passing grains.

Once *Ranunculus* biomass decreases then the unidirectional flow of streams applies pressure to the plant in one direction causing compression of the plant tissues. This leads to a dense plant tissue matrix that deflects flows around the plant instead of through the stand. Indeed, maximum shear stress for a number of macrophyte species is typically focussed at the canopy surface (Sand-Jensen & Mebus, 1996). Thus for particles suspended in the water column there may be very little exchange with the water within the plant stand. Therefore organic particles in suspension may have limited opportunities to enter macrophyte stands and so will not experience the low flow areas within the stand that promote the deposition of fine particles.

Sand-Jensen & Pederson (1999) found that reaches with abundant *Ranunculus* stands had higher turbulence values around the macrophytes then for any of the other macrophyte species studied. The increased turbulence was attributed to the large biomass structures that these macrophytes develop. These cause large-scale deflection of flows around the stands causing rapid acceleration and deceleration of the flow. In

addition, the long trailing sections of the *Ranunculus* stands have strong wave motion that can cause eddies, further increasing turbulence (Sand-Jensen & Pederson, 1999).

The principal impact of late-season *Ranunculus* may be to increase water levels and thus decrease contact with alternative trapping devices, such as cobbles and woody debris. Reynolds et al. (1990) released *Lycopodium* spores in a flume, which allowed flow conditions to be manipulated to determine the primary controls on particle deposition. It was found that water depth, and not water velocity, principally controlled the deposition of the *Lycopodium*. Water depth was also found to have a positive effect on mean transport distance for CPOM (Brookshire & Dwire, 2003). Wanner & Pusch (2001) found that macrophyte beds in the River Spree were poor at trapping *Lycopodium* spores that were released into the stream as an analogue organic particle. They did however find that the macrophyte stands were efficient stores of organic matter and reasoned that although poor trappers of material, once the material was trapped the protective nature of the macrophyte stand prevented the material from being released back into the river.

The data presented above show variation in the degree of particle transport over time within chalk streams. However comparing the V_{dep} values against other river systems allows the relative trapping efficiency of the systems to be compared. Values in the literature show chalk stream systems to be highly retentive in comparison with other systems. Georgian *et al.* (2003) gave V_{dep} values of between 0.313 and 0.094, however, these values were for streams with a channel slope of between 0.14 – 0.051 m m^{-1}, while calculations based on the data presented by Miller and Georgian (1992) show V_{dep} values of 0.256 and 0.206 in a stream with a mean gradient of 0.013 m m^{-1}. In comparison channel slope values for chalk streams are of an order of 0.001 m m^{-1}

(Hearne & Armitage, 1993). Georgian *et al.* (2003) refers to an unpublished MS Thesis by Ehrman who recorded a greater range of V_{dep} values for corn pollen. The highest value recorded was 1.8, a higher value than was seen in the Bere Stream. These differences were attributed to Ehrman studying a wider range of streams than those studied by the previous authors.

The differences in the transport capabilities between the two large scale *Ranunculus*-free reaches was also interesting. The *Ranunculus*-free reach in June was well established as a macrophyte-free reach as there was a mature wood shading the reach and had a substratum consisting predominantly of large cobbles. In contrast the removal of the macrophytes in July left a substratum that consisted of much finer material than in the June *Ranunculus*-free reach. The low discharge found in chalk streams in summer did not have the power to wash this material out of the reach even when the protective cover of the macrophytes was removed. This material would probably not be removed until the high winter discharges occur. This difference in the substratum of the two reaches may explain in part the differences in the retention capabilities of the two reaches (see section 5.3 below) and demonstrates the heterogeneity of these streams even after the removal of the macrophytes.

5.3 Influence of the hyporheic zone on particle transport

Studies on the transport of organic matter in streams typically focus on the role of surface features in the trapping and storage of particles. Comparatively little work has been done on the impacts of fine inorganic sediments on interstitial organic matter. However in many systems the trapping and subsequent storage of organic material in the hyporheic zone can exceed that stored on surface features by several orders of magnitude, due to lower decomposition rates and reduced downstream transport. (Metzler & Smock, 1990; Valett et al., 1990). A study on two gravel bed rivers in Europe found that although inorganic particles of less then 1mm formed only 6 – 9% of the total sediment, as much as 88% of total organic carbon and nitrogen were associated with this material (Brunke, 1999). Thus these particles are likely to have a significant role on river processes and will impact on organic matter processing in these systems.

There is an increased need to elucidate the interaction between fine inorganic sediments and organic particles within chalk streams. Recent increases in the quantities of fine sediments have been noted in chalk streams across England. These are manifest as increased turbidity and large deposits of fine sediments on the surface of the stream substratum (Walling & Amos, 1999). This retention of fine particles in river systems, colmation, occurs both on the surface, or within the gravels, of the hyporheic zone. This can lead to clogging of the river sediments and restrictions on the exchange of surface waters with deeper groundwaters. These deposits will remain until discharge increases and the fine sediments are flushed from the system, decolmation, leaving the larger gravels behind (Brunke, 1999). The distinctive well-attenuated flows associated with chalk streams is likely to ensure that colmation will occur throughout the summer as discharge decreases and that decolmation events only occur following re-charge of the chalk aquifer and an increase in stream discharge.

The accumulation of fine sediments has both direct and indirect effects on the ecology of rivers. Direct effects include a reduction in pore space within the hyporheic zone and decreased hydrological exchange between hyporheic and surface waters. This may then lead to indirect effects such as changes to the physico-chemical characteristics of the hyporheic zone as the residence time of the water is increased (Olsen & Townsend, 2003).

5.3.1 Flume methods

The current study aimed to determine the impact that the addition of fine inorganic sediments to a gravel bed stream had on the transport of organic particles (corn pollen) through a reach. Due to the methodological difficulties involved in manipulating the hyporheic zone in natural rivers a flume was used to enable the substratum material to be controlled. The flumes are based at the River Laboratory, East Stoke, Dorset, and receive a constant flow of stream water from an old mill channel that has been diverted away from the main channel of the River Frome. The flumes consist of two parallel 1.4 m wide by 7 m long channels, the sides of the channels have glass windows to allow observations into the flume. The entrance to each flume is controlled by a sliding door and this provides some control over the discharge travelling through each flume.

To determine the influence of fine inorganic sediments on the trapping of organic particles a series of releases were undertaken with two different substratum types in the flume. For the first treatment the flume was filled to a depth of 15 cm with two tonnes of cobbles to simulate the gravel beds seen in chalk streams. To replicate the affects of fine sediment accumulation on organic particle transport approximately 500 kg of sharp sand was added to the flume in order to infill the interstitial gaps within the gravel bed for the second treatment.

To develop a clearer understanding of the passage of water through the flume a conservative tracer, NaCl, was added. A total of 5 kg of NaCl was added to 25 L of stream water and stirred until fully dissolved. The tracer was released, as a gulp, 20 m upstream of the entrance to the flume. The channel splits into two when it reaches the flume so the tracer was released directly in front of the flume with the gravel treatments to ensure that most of the tracer flowed through the flume under investigation and not through the second flume. Conductivity readings were taken just inside the entrance to the flume every 5 seconds using a conductivity meter, until readings returned to background.

Corn pollen was used as a faecal pellet analogue, staining, release, collection and processing of the pollen was identical to the techniques used for the macrophytes (see chapter 5.2.1), 1 g of corn pollen was released on each occasion and samples were collected at the upstream entrance of the flume and the downstream end of the flume just before the water exited the flume. Based on the NaCl tracer data the sampling for the corn pollen started at 40 s with samples taken every 20 s until 4 min after the release of the sample. After the release a note was made of the water level in the flume and the system was left overnight so that any corn pollen trapped was washed out of the system. The following day the sand was added and the release was repeated using the same technique as before, the only difference being that the first sample was taken at 45 s and then every 20 s afterwards.

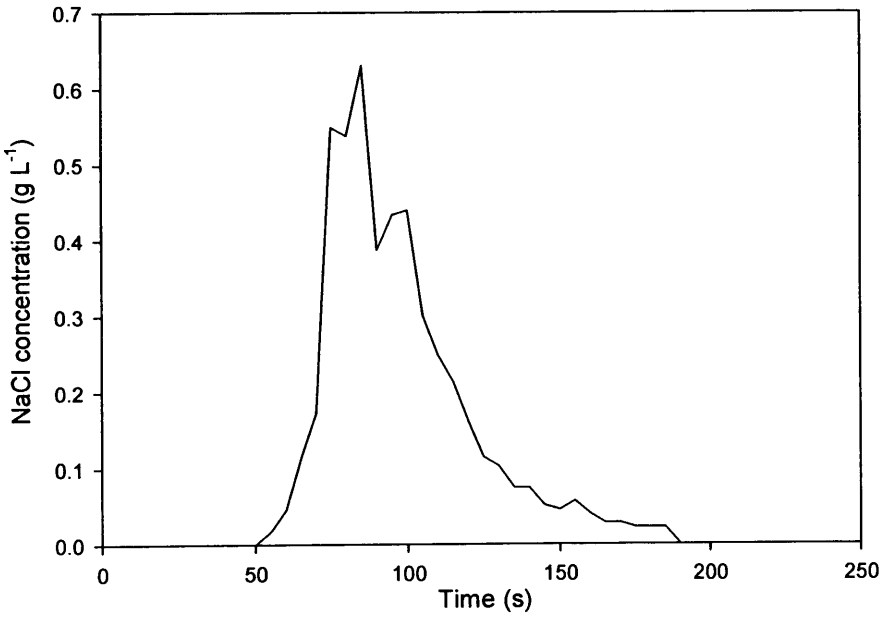
This experiment was repeated again in September 2004, the methods were essentially the same as above, however, to gain a clearer resolution on the transport of the pollen grains the sampling started after 20 s and the sampling interval was halved to 10 s. The

downstream sampling site was also moved further downstream to immediately outside of the flume which allowed better mixing of the water as it left the flume.

5.3.2 Results

The NaCl tracer release conducted prior to the release of the pollen grains determined the sampling intervals for the corn pollen. Conductivity values started to increase at 55 s post release and returned to background after 190 s post release. These values were transformed to NaCl concentration after testing using standards to determine the relationship between conductivity and NaCl concentration ($y = 1726x + 491$) (see chapter 6.2.1) (Figure 5.16).

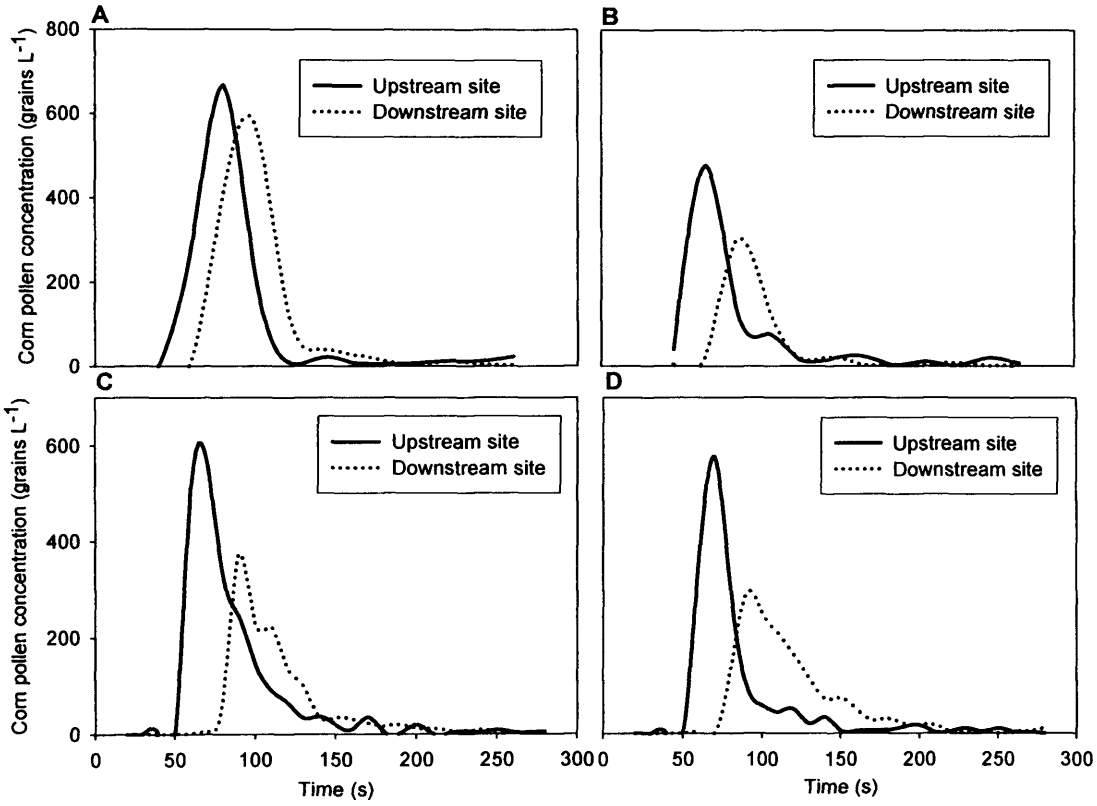
Figure 5.16; NaCl concentration - time curves upstream of the flume, 29/06/04, these data were used to determine the sampling intervals for the corn pollen releases.



Corn pollen concentration – time curves were constructed for the upstream and downstream sites to show the pattern of transport of pollen grains as they were transported through the flume. By integrating the area under the curves it was possible

to calculate number of grains that passed through the sampling point and the difference between the two would show the retention efficiency of the flume (Figure 5.17 & Table 5.2).

Figure 5.17; Corn pollen concentration – time curves; June 2004, A = gravels only & B = gravels & sand; September 2004, C = gravels only & D = gravels & sand.



For the release on the June 2004, 2 % of the corn pollen was trapped within the reach when only gravels were present in the flume, the addition of the sand caused retention to increase to 34%. On the September 2004, corn pollen retention was 9% with only gravels present and increased to 37% retention when both gravel and sand were present.

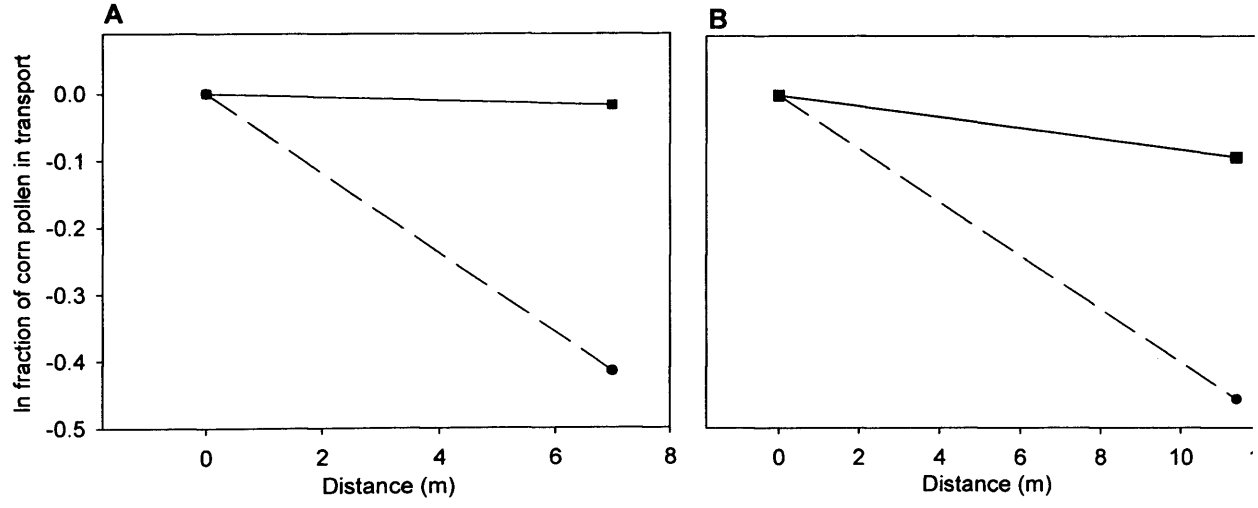
The loss rate of the corn pollen (K_L) with only gravels in the flume was an order of magnitude lower (-0.0029 & -0.0082) than when the sand was added (-0.0594 & -0.0405) (Figure 5.19). The mean transport distance (S_W) of the corn pollen for the 2004 releases declined from 345 & 121 m to 17 & 25 m. The V_{dep} values for the releases

showed the gravel treatments to have values of 0.46 in June and 1.13 in September and much higher values of 9.34 and 5.59 for the gravel and sand treatments (Table 5.2). The difference between the values for the gravel and sand treatments was primarily due to the change in length between the sampling sites from June to September 2004.

Table 5.2; Summary of results from the release of corn pollen in flume.

	June 2004		September 2004	
	Upstr.	Downstr.	Upstr.	Downstr.
Grains in transport (Gravel)	5,497,360	5,402,980	3,068,296	2,796,024
Grains in transport (Gravel & sand)	3,544,640	2,348,280	3,996,564	2,529,923
% CP retained (Gravel)	2		9	
% CP retained (Gravel & sand)	34		37	
Width (m)	1.4		1.4	
Mean Depth (m)	0.31		0.27	
Reach distance (m)	7		11.4	
Mean velocity ($m\ s^{-1}$)	0.507		0.519	
Discharge ($m^3\ s^{-1}$)	0.22		0.187	
K_L; (Gravel)	-0.0029		-0.0082	
K_L; (Gravel & sand)	-0.0594		-0.0405	
Estimated S_W (Gravel)	345		121	
Estimated S_W (Gravel & sand)	17		25	
V_{dep} (Gravel)	0.46		1.13	
V_{dep} (Gravel & sand)	9.34		5.59	

Figure 5.19; loss rate of corn pollen from the water column, calculated as the slope of the line when the natural-logarithm of the fraction of corn pollen in transport is plotted against distance. A = June 2004, B = September 2004; Solid lines = gravel only; Dashed line = gravel and sand.



Attempts were made to calculate the Nominal Transport Time (NTT) through the reach using NaCl releases, however the short length of the flume meant that the differences in the transport characteristics of the two treatments were impossible to determine.

Instead the flume characteristics were determined using an electromagnetic flowmeter to calculate discharge and mean velocity. These showed the mean depth of the flumes to be within the range seen in the macrophyte releases although the mean velocity in the flumes were higher (>0.5 m s^{-1}) than those recorded in the macrophyte reaches which were typically less than 0.3 m s^{-1}. The flumes had a discharge of 0.22 m^3 s^{-1} in June and 0.187 m^3 s^{-1} in September.

5.3.3 Discussion

The addition of the sand to the flume promoted the substantial retention of corn pollen, creating very high deposition rates as comparison of the flume V_{dep} values with those for the macrophyte removal demonstrates. The V_{dep} values for the gravel and sand are the highest recorded in the literature and probably a result of the relatively large area of the channel cross-section that is filled by the gravel/sand matrix. Channel depth in the flume was around 30 cm while the depth of the gravel and sand was 15 cm leading to substantial proportion of the surface water interacting with the substratum. This may have led to an exaggerated effect for the trapping ability of the fine sediments in natural streams.

Previous studies have suggested that streams with large quantities of fine sediments act as poor trappers of organic particulates as they are easily eroded so releasing the trapped material. Thus larger structural elements are needed within the channel to trap the material and store it long enough to be processed with the stream (Koetsier & McArthur, 2000). However, the ability of sand to trap large quantities of particulates

from water flowing through the sand has been appreciated in the water industry as the wide-spread use of slow sand filters demonstrates (Wotton, 2002). Therefore the primary determinant on the ability of fine inorganic sediments to trap particles is likely to be the degree of interaction between the particles and sand.

In gravel bed rivers, such as chalk streams, bed material can be divided into two compartments. The larger gravels that form the 'framework' of the bed and the 'fines', the fine inorganic particles that infill the framework. It is the latter that impact most on the hydraulic conductivity of the hyporheic zone. As the gravels are infilled with fines then there is a reduction in pore space leading to increased sieving and trapping of particulates as they are transported through the pore water (Brunke, 1999). This was clearly demonstrated by the effect that the addition of fine inorganic particles to the gravel framework in the flume had on the transport of the corn pollen.

Variations in the topography of river beds create pressure gradients that induce exchange of surface waters with the hyporheic zone. Particles within the boundary layer flows are then transported into the sediments where they are trapped (Huettel et al., 1996). Thus even in a relatively homogeneous environment such as that found in the flume there is enough variation in the topography to induce the downward transport of corn pollen into the sediments.

The dominant process causing the retention of the corn pollen in the flume is through mechanical filtration by the fine sediments. For smaller particles of less then 30 μm physico-chemical filtration becomes the dominant process (Brunke, 1999). As fine sediments are trapped they form deposits that in turn act to promote the trapping of particles leading to a positive feedback process that will increase particle trapping (Huettel et al., 1996). These organic-rich deposits will be sites of intense biological

activity that will lead to increased microbial activity and become covered in biofilms. Biofilms produce exopolymers that trap and bind particulate matter through physico-chemical filtration leading to further colmation and reducing the permeability of the sediments (Huettel et al., 1996; Pusch et al., 1998; Brunke, 1999). The trapping of organic particles by fine sediments in chalk stream reduces nutrient spiralling length as particles become trapped. In areas of the hyporheic zone with anaerobic conditions the subsequent processing of this material will be greatly decreased (Metzler & Smock, 1990).

While sand-bottomed streams have lower surface storage then streams with coarser substratum, the bed is more dynamic with high discharges causing the periodic release and burying of material as the substratum is eroded (Metzler & Smock, 1990). This is unlikely to be the case in chalk streams where the sand has filled in the pore spaces between coarser gravels. These gravels provide a rigid framework that acts to stabilise the sand deposits. The stability of gravels is compounded by the relatively stable hydraulic conditions that prevail in chalk streams. Therefore, particulates trapped within the hyporheic zone are unlikely to be released through scouring of the sediments and are relatively well protected.

The trapping of organic particulates by fine sediments will influence the metabolism of the hyporheic zone as it is dependent on the importation of organic material from surface waters. Indeed the community respiration of hyporheic sediments and the invertebrate community have been shown to be highly correlated to the quantity of organic particulates trapped within the sediments (Lentings et al., 1997; Crenshaw et al., 2002).

5.4 Conclusions

The use of corn pollen as an organic particle tracer has helped to elucidate some of the processes that impact on faecal pellet transport within chalk streams. These show the importance that surface features such as macrophytes can have in trapping FPOM and the temporal variation that macrophyte life cycles impose on transport. Additionally, the tracers have shown the importance that bed sediment size can play on the transport of particulates.

Although these experiments have sought to try and isolate the factors that control particle transport there is considerable interaction between these two components. Within a chalk stream reach with substantial macrophyte growth there will be patches of active colmation and decolmation as the macrophytes impact directly on flows and therefore indirectly on fine sediment distribution. These discrete patches of fine sediments will, in turn, further impact on the transport and storage of FPOM within the stream.

It is likely that macrophyte-dominated streams create conditions that favour the interaction of suspended FPOM with subsurface sediments. A significant control on the exchange of surface waters with groundwaters are longitudinal changes in cross-sectional areas. These changes generate pressure gradients between surface and groundwaters leading to zones of upwelling and downwelling (Pusch et al., 1998). The patterns of macrophyte growth in chalk streams lead to localised areas of channel constriction as seen by increased flows and clean gravels that provide the conditions necessary for water interactions.

The influence of macrophytes on subsurface flows leads to changes in the nutrient status of the hyporheic water which then influences the growth of aquatic macrophytes in the

surface waters above (Valett et al., 1990). Hendricks and White (1988) found that macrophytes altered flow patterns into the hyporheic zone in a functionally similar manner as other surface features such as riffles and woody debris. *Ranunculus* stands have been shown to promote downwelling at Bere Stream, following removal of the *Ranunculus* these areas then revert to an upwelling state (I. Sanders; pers. comm.). These data suggest that macrophytes are responsible for forcing surface waters and associated FPOM down into the hyporheic zone, where they will then come into contact with the fine sediments associated with macrophytes leading to enhanced retention of these organic particles within the system.

The use of tracers to clarify the factors that impact on FPOM dynamics have shown the potential to help answer some of the questions in this area. However, experimental design requires careful consideration. The use of flumes allows variables that may be important to be isolated and for replication of results. The use of flumes though does lead to questions on the applicability of the findings to natural channels.

Using tracers in natural streams overcomes this concern but it becomes harder to isolate the particular variable being studied. There are also logistical and consent issues relating to the manipulation of natural streams that have to be considered. Another key concern of natural channel experiments relate to replication. For example to replicate the macrophyte removal experiments would require having a series of reaches with approximately similar conditions. Overcoming these hurdles is feasible and should allow some of the questions that have been answered for CPOM transport to be answered for FPOM.

6 Factors influencing the degradation of blackfly larvae faecal pellets

6.1 Introduction

The binding mechanism of faecal pellets is central to the formation and persistence of these aggregates within the stream environment. Some invertebrates produce faecal pellets that are wrapped in a peritrophic membrane, these membranes line the gut and provide mechanical and pathogen protection to the midgut epithelium from the ingested food (Lehane, 1997). Upon egestion of the faecal pellets the small pore apertures of the membranes prevents immediate invasion of the pellets by free-living bacteria (Lampitt et al., 1990). After colonising the surface of the pellet bacteria eventually succeed in rupturing the membrane leading to loss of material through the rupture point (Hansen et al., 1996). Copepod faecal pellets have been shown to require coprorhexy (physical disruption of the pellets by organisms) before they can be colonised by microbial organisms (Gonzalez & Biddanda, 1990).

Invertebrates commonly use exopolymers (exudates containing polysaccharide chains that hydrate in the presence of water) to bind together the constituent particles and so maintain the cohesion of their faecal pellets (Wotton, 2004b). The exopolymers used in the binding of faecal pellets are acquired by the organism either through ingestion from the environment or are produced by live algae and bacteria that are contained within the gut (Wotton, 2004a; Wotton, 2004b). The binding mechanism of faecal pellets is important as this maintains the integrity and distinctive characteristics of faecal pellets with the environment and to a large extent determines the transport and fate of both the individual particles within the pellet and of the pellet as a whole.

Herbivorous sea urchins grazing on algae in marine benthic environments have a low assimilation efficiency and much of the material they ingest is excreted as faecal pellets. These faecal pellet deposits form a nutritious food source that is utilised by detritus feeders (Mamelona & Pelletier, 2004) and are analogous to the extensive faecal pellet deposits seen in chalk streams (see chapter 3 above). As well as ingestion by consumers faecal pellet deposits represent a resource that can be utilised directly by the microbial community. Decomposition of organic matter in streams is dominated by bacteria and fungi. Much of this work has been based on leaf degradation and shows fungi to play a key role early in the degradation processes, as their vegetative hyphae are capable of invading and breaking down the leaf material, while bacterial activity is typically restricted to the leaf surface and increases later in the degradation process as the surface area of the material increases (Schlickeisen et al., 2003). Faecal pellets will have a high bioavailability to microbes, leading to mineralisation and enhanced nutrient release to the overlying water column as the pellets are degraded (Kautsky & Evans, 1987; La Rosa et al., 2002; Giles & Pilditch, 2004). In turn the microbial community associated with the pellets can represent an important food source for macroinvertebrates that will graze on the microbes (Edwards & Meyer, 1990).

Bivalve aggregations produce large quantities of faecal pellets and pseudofaeces which sink to the substratum forming extensive biodeposits (Giles & Pilditch, 2004). The degradation of bivalve faecal pellets occurs in two main stages. A preliminary stage, where material is lost through hydrolysis, is mediated by bacteria that have survived passage through the bivalve tract. The second step is a longer process that involves the breakdown of the more refractory material within the pellet. It is thought that the second stage is regulated primarily through environmental conditions, notably temperature (Grenz et al., 1990). It has been hypothesised that the microbial community in faecal

pellets can come from two primary sources. After excretion pellets can be colonised by free-living bacteria from the water column which colonise the surface of the pellets (Hansen et al., 1996). Alternatively, the bacteria can be intrinsic to the pellets having survived passage through the gut of the organism (Grenz et al., 1990), however the relative importance of the two sources is not clear (Lampitt et al., 1990). As faecal pellets are colonised and degraded by microorganisms the carbon to nitrogen ratio decreases as the microbes utilise the carbon within the pellet for respiration (Stuart et al., 1982). The C:N ratio is an indicator of food quality, as C:N values of a material decrease the food quality of the material increases (Kautsky & Evans, 1987).

This study aimed to determine the importance of peritrophic membranes and exopolymers to the binding of particles contained within faecal pellets. The source of the microorganisms responsible for the degradation of the faecal pellets will also be determined. It is hypothesised that the low assimilation efficiency of blackflies (Wotton, 1978) will allow some bacteria to survive transit through the gut, coupled with the dense nature of the faecal pellets this will favour microorganisms intrinsic to the pellets to dominate the degradation process. The impact of environmental temperature on faecal pellet degradation will also be investigated, to determine if faecal pellets incubated at a higher temperature degrade at a faster rate than those kept at a lower temperature. Finally, variation in the C:N ratios over time will be determined. Blackfly faecal pellets will be expected to show a decrease in their C:N values over time as they are colonised by microorganisims.

6.2 Methods

6.2.1 Determining the binding mechanism in faecal pellets

To understand how the constituent particles of the faecal pellets are bound together, histological stains were used to distinguish the material responsible for the binding. To determine if the pellets are held together by a peritrophic membrane, a predominantly chitin structure (Lampitt et al., 1990), pellets were stained with Calcofluor which binds strongly to chitin (Horobin, 2002). The staining solution consisted of Calcofluor dissolved into distilled water at a concentration of 1 mg ml^{-1}. Freshly produced blackfly faecal pellets were placed into the stain and left for 5 minutes to allow uptake of the Calcofluor (P. Joyce pers comm.). Peritrophic membranes are produced in the gut of invertebrates and to ensure that the peritrophic membrane was being stained the gut contents of a blackfly larva were dissected out and stained with Calcofluor. After staining the pellets and guts were placed into enamel trays filled with distilled water to remove excess stain before mounting them onto glass microscope slides. These were placed under a Nikon Axioskop II microscope and illuminated using a mercury vapour light source, which caused the Calcofluor to fluoresce strongly at an excitation wavelength of 380 nm with an emission wavelength of 425 nm. Images were collected using a Hamamatsu Orca Research Grade digital camera.

To determine if the pellets contained large quantities of exopolymers they were stained with Alcian Blue, which binds tightly to exopolymers (Malmqvist et al., 2001). Pellets were covered with Alcian Blue solution and left for 5 minutes to allow uptake of the stain (P. Joyce pers comm.). Pellets were then placed into enamel trays filled with distilled water to remove excess stain before being examined under a dissecting microscope to determine the presence of exopolymers, which stained bright blue.

6.2.2 Effect of temperature on the conditioning of faecal pellets

Faecal pellets were incubated at a range of temperatures to determine if the ambient environmental temperature has an effect on their conditioning. A number of variables can be measured as surrogate measurements of conditioning. Experiments looking at leaf degradation frequently measure the loss of mass of leaf material over time (Schlickeisen et al., 2003). These experiments are standardised by using leaf discs with a known initial area and dry mass from which subsequent mass losses can be compared under different treatments. These techniques were not suitable for use with faecal pellets due to the difficulties associated with the acquisition and manipulation of pellets. Changes to the surface area of the pellets was selected as the best variable to record as it is both non-destructive and does not require moving the pellets. Faecal pellets are an aggregation of much smaller particles and as the they degrade they disaggregate (Taghon et al., 1984) leading to an increase in apparent surface area. Pellets also provide a substrate for microbial growth and the incorporation of microbial biomass will act to further increase the surface area of the pellets, while swelling has been recorded for pellets produced by copepods in marine environments (Hansen et al., 1996).

The pellets were incubated in micro-plate observation trays with 1 cm diameter wells. These trays have good optical quality enabling repeat images of the pellets to be made over time. Initial trials showed that individual pellets were too small to record accurate differences in their surface area and therefore pellets were placed in groups of 15. This number of pellets magnified the effect of changes in apparent surface area and was measurable using the available image analysis software. This technique has been used previously to determine the surface area of pseudofaeces produced by bivalves (Giles & Pilditch, 2004).

Pellets were harvested from larvae fed on fresh stream water using the techniques described in section 4.2 above, this ensured that the pellets were fresh and of a known age before the initiation of the experiment. Faecal pellets were incubated at four different temperatures of 4, 10, 15 and 22°C in batches of 15 pellets per well, with three replicates for each treatment. The choice of temperatures was determined by the availability of incubators but covered that seen in natural chalk streams. As the surface area of the pellets, as measured by the image analysis software, is dependent on the orientation of the pellets, it was not possible to change the water within the wells without disturbing the pellets so the micro-plate trays were covered to reduce evaporation.

To record the faecal pellet images the micro-plates were placed on top of a lightbox, backlighting the pellets in this manner gave a clear silhouette allowing the apparent surface areas of the pellets (the surface area of the silhouette) to be determined. Images were captured with a JVC TK-C921EG digital color video camera, mounted above the lightbox. Images were taken immediately after the pellets were first placed into the wells and this represented day 0 and the baseline from which all subsequent differences in surface area were compared. Images were taken twice a week, although on occasions this was not always possible, and continued until microbial growth within the wells prevented clear images of the pellets being taken. The pictures were analysed with ImageJ, a public domain Java image-processing programme. To calibrate the computer programme the length of an iron filing was measured under a dissecting microscope using a calibrated eyepiece micrometer. The filing was then placed into a well, covered with water and an image of the filing taken using the technique described above. The length of the filing was put into ImageJ, allowing unit area per pixel to be determined.

This scale was used for all measurements of the faecal pellet surface area that were subsequently taken.

6.2.3 The influence of microorganisms on the degradation of faecal pellets

To determine the impacts that microorganisms play in the degradation of faecal pellets a series of four treatments were set up. The treatments were designed to interpret the relative importance of microorganisms from different origins to the conditioning of faecal pellets.

Treatment A – Fresh faecal pellets incubated in fresh stream water (FPs + SW). An indicator of the natural degradation of faecal pellets.

Treatment B – Fresh faecal pellets incubated in autoclaved stream water (FPs + AC SW). Will show the importance of microorganisms contained within the faecal pellets.

Treatment C – Autoclaved faecal pellets incubated in fresh streamwater (AC FPs + SW). Demonstrates the role of microorganisms contained within the water column.

Treatment D – Autoclaved faecal pellets and autoclaved streamwater and gentamicin (AC FPs + AC SW + G). Will inhibit all microbial growth and show what happens when there is no microbial component to degradation.

Autoclaving is an effective and well-established technique used to sterilise material, trials indicated that autoclaving did not cause disruption to the pellets and this was chosen as the most effective way to kill all microorganisms in the samples. Gentamicin was added to the treatments at a concentration of 2.5×10^{-5} g ml^{-1} was used as it has an inhibitory effect on the growth and development of microorganisms (Wotton et al.,

1997) and would maintain sterile conditions within the wells preventing recolonisation of the autoclaved material by microorganisms from the surrounding environment.

The methods for collecting and processing the images was identical to that used in the trial on the effects of temperature on faecal pellet conditioning (see 6.2.2 above). This experiment was repeated twice. The first experiment was incubated at 15°C, this is a higher temperature than that typically found within chalk streams although within the range found during the summer10-15°C (Westlake et al., 1972), however at the time of the experiment this was the closest temperature available. The micro-plate had been used previously and was sterilised using ethanol prior to the establishment of this experiment. However the sterilisation process was not effective and there was extensive microbial growth that caused the experiment to finish early necessitating repetition of the experiment. Experiment 2 was established using the same treatments as experiment 1, but with some key differences. The tray was incubated at 10°C as an incubator at this temperature was available and a new sterilised micro-plate was used to reduce the influence of external microorganisms.

6.2.4 The C:N value of faecal pellets

The carbon to nitrogen ratios of faecal pellets were determined using a CE440 Elemental Analyser (Exeter Analytical, Uxbridge, UK). The machine required a minimum of 1.7 mg of material to record the ratio (G. Maxwell. pers. comm.). Trials indicated that it was possible to collect and incubate a suitable quantity of faecal pellets that would allow repeat measures of the pellets over time to be made. To produce enough faecal pellets for the experiment larvae and stream water were collected from the River Chess. These were divided into three buckets and left for 24 hours to allow the larvae time to produce enough faecal pellets. The pellets were collected from each

bucket and placed into 350 ml glass jars with 200 ml of fresh stream water, this provided three jars of pellets for replication (for a full description of the techniques used for the production and collecting of the faecal pellets see section 4.2 above). Microorganisms on the surface of the pellets require nutrients from the water column and therefore the stream water in the jars was changed twice-weekly. Pellets were sampled weekly, a Pasteur pipette was used to draw up faecal pellets from the jar and these were placed into glass sample tubes. This process was repeated until a suitable quantity of material was in the tubes (determined visually but based on trials). The tubes were placed in an oven at 105°C and left overnight to dry and then frozen until ready to be tested.

6.3 Results

6.3.1 The structure of faecal pellets

When the blackfly larva gut was illuminated using a mercury vapour light source the Calcofluor stain clearly showed the presence of a peritrophic membrane surrounding the ingested material (Figure 6.1A). Backlighting with a conventional light source reveals the contents of the gut within the peritrophic membrane (Figure 6.1B). It is interesting to note the position of the rupture in the membrane wall as this corresponds with the loss of gut material through the rupture point in the backlit image. The gut content appears to be unconsolidated and of a very different nature to the blackfly faecal pellets which are found within the stream environment that maintain their physical integrity even though they are not constrained in any way by external membranes or similar structures. Images of Calcofluor stained blackfly guts and faecal pellets were taken using the same camera exposure to reveal differences in the uptake of Calcofluor between the two materials. These show the guts to fluoresce very brightly (Figure 6.2A) while the faecal pellets barely fluoresce at all, although very slight fluorescing can be seen in the centre of the image, which may be small pieces of peritrophic membrane bound within the faecal pellet (Figure 6.2B).

Figure 6.1; Images of a Calcofluor stained blackfly larva gut content. A) Peritrophic membrane illuminated by a mercury vapour bulb. B) Gut illuminated by standard backlighting showing the contents of the gut.

A B

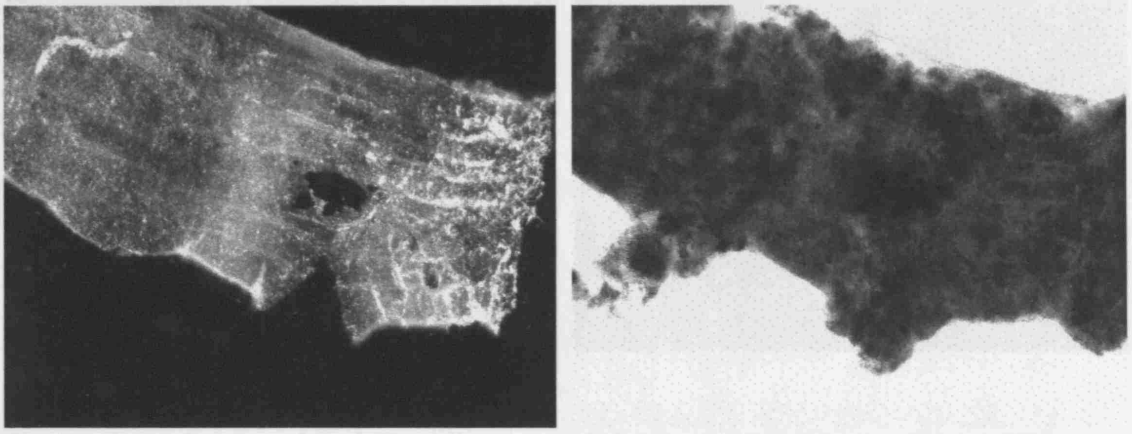


Figure 6.2; Images of Calcofluor stained larva gut (A) and faecal pellet (B) taken with the same exposure. Some slight fluorescing can be seen in the faecal pellets image.

A B

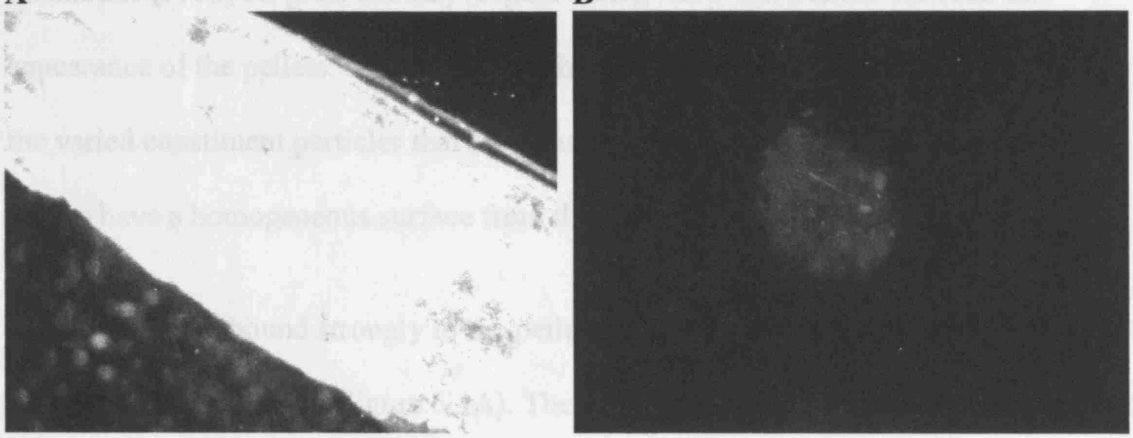


Figure 6.3; Faecal pellets from A) blackfly larva and B) *Gammarus pulex*

A B

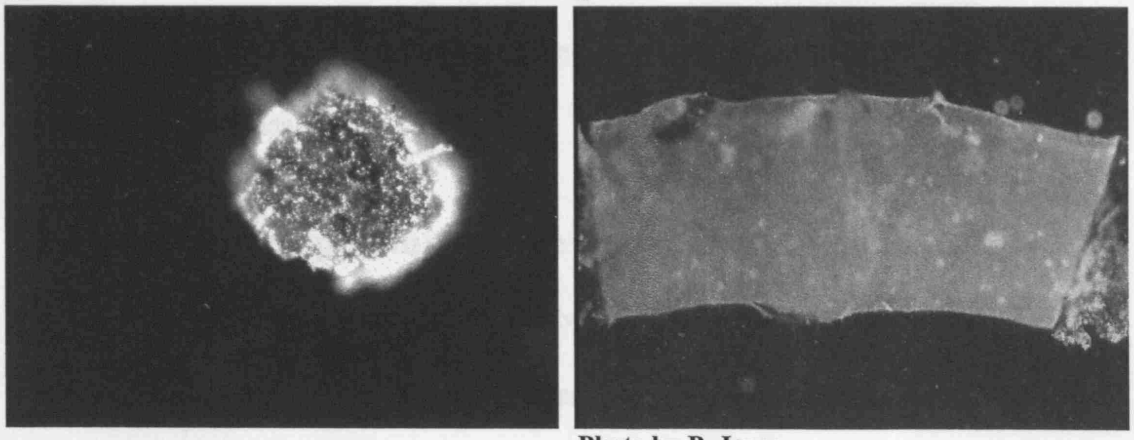
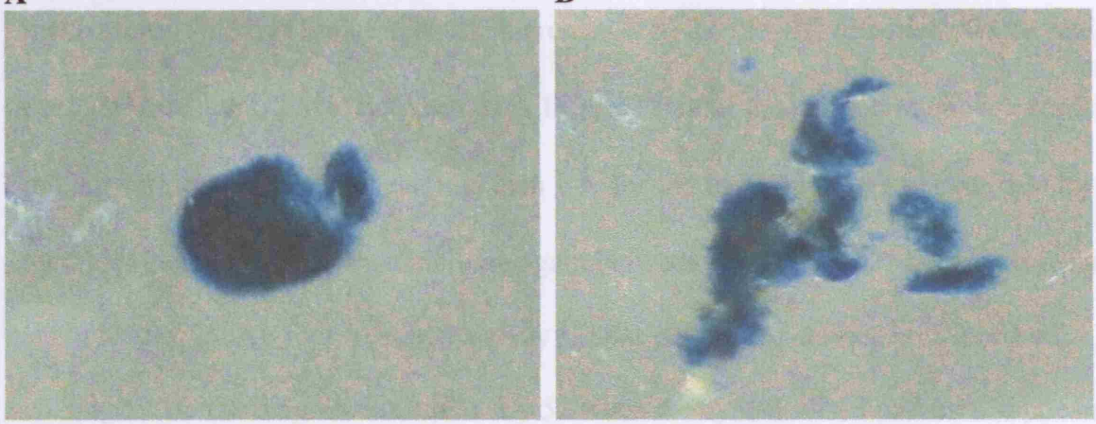


Photo by P. Joyce

Figure 6.4; Alcian Blue stained pellets, A) whole pellet, B) disrupted pellet.

A **B**



Comparisons between blackfly pellets, containing no peritrophic membrane (Figure 6.3A), with faecal pellets of *Gammarus pulex*, which are encased in peritrophic membrane (P. Joyce, pers. comm.) (Figure 6.3B), show differences between the appearance of the pellets. Blackfly pellets have a heterogeneous surface as a result of the varied constituent particles that form the faecal pellet, whereas the *Gammarus* pellets have a homogeneous surface from the peritrophic membrane.

The Alcian Blue bound strongly to the pellets indicating abundant exopolymers being present within the pellet (Figure 6.4A). The exopolymers are evenly distributed forming a constant blue matrix around the pellets. The pellets were disaggregated using a dissecting needle and this showed the Alcian Blue to have permeated into the pellet and provides evidence that the exopolymers are the principal agent that binds the ingested material into the faecal pellet.

6.3.2 Effects of temperature on the conditioning of faecal pellets

The conditioning of the faecal pellets incubated at different temperatures showed a similar pattern in all of the treatments. After three days of incubation all of the faecal pellets showed a decrease in apparent surface area of between 12 and 17 %. From day three to day 14 there is a rise in apparent surface area for all of the treatments. The 10

and 15°C treatments had surface areas that were over 30 % larger than at the start of the incubations. This was higher than the increases seen for the 4°C treatment, 23 %, and the 22°C treatment, 27 %. Between days 14 and 17 all of the treatments show a decrease in their surface areas of between less than 1 % for the 15°C treatment to over 6 % for the 22°C treatment. They then increase again between days 17 and 21 before decreasing again by day 25. All four treatments show similar patterns of surface area increase, although different magnitudes, up to day 52 when the synchronised pattern disappears. The 4°C treatment shows a trend of decreasing size so that by the end of the experiment (day 66) the pellets have increased by 21 % compared to their original size. The 10 and 15°C treatments are both over 40% larger than their original size while the 22°C treatment is over 60% larger (Figure 6.5).

To determine if temperature caused significant differences in the % increase in surface area of faecal pellets the data were analysised using a Kruskal-Wallis test. This showed there to be significant differences between the treatments ($P = 0.029$). Mann-Whitney tests were used to identify pair-wise differences in the % increase in the surface area of the faecal pellets. These showed the faecal pellets incubated at 4°C were significantly different from the pellets incubated at 10 and 15°C, none of the other treatments were significantly different from each other (Table 6.1).

Figure 6.5; The effect of temperature on the conditioning of faecal pellets at A = 4°C, B = 10°C, C = 15°C and D = 22°C. Error bars = 95% confidence intervals.

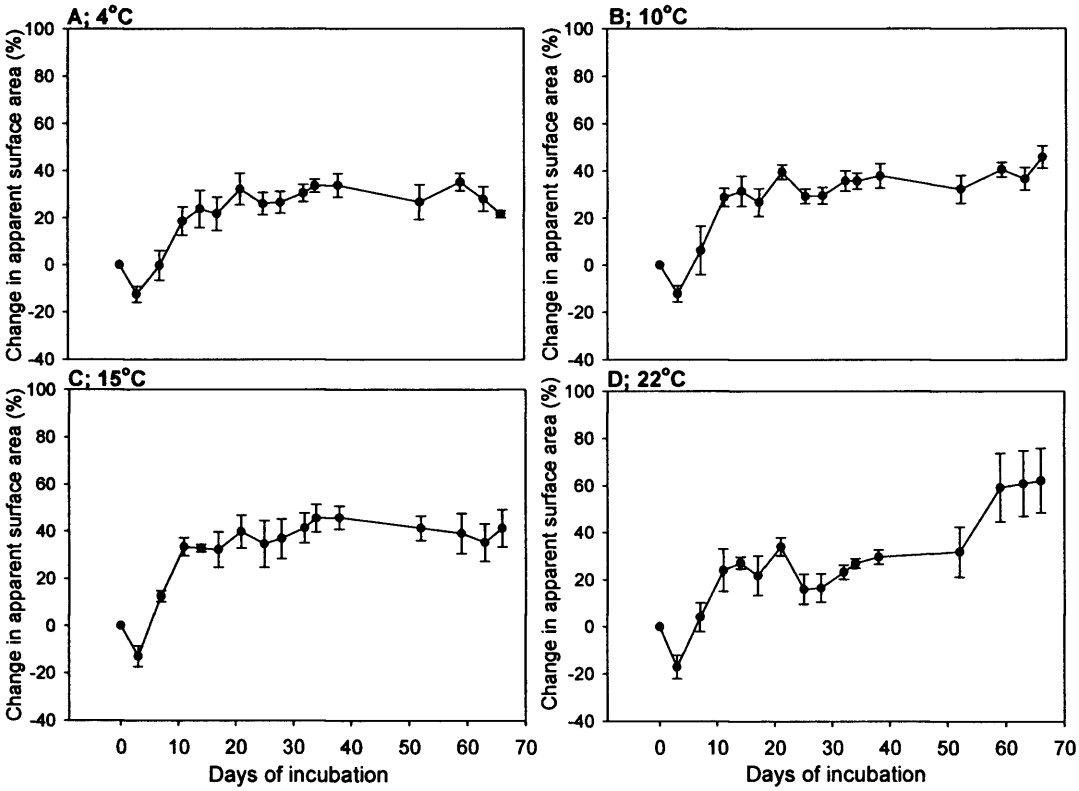


Table 6.1; Results of Mann Whitney tests showing significant differences in the increase in size of faecal pellets incubated at different temperatures. Asterisks represent P values, * P = <0.05, ** P = <0.01, *** P = < 0.001.

10oC	*		
15oC	**		
22oC			
	4oC	10oC	15oC

6.3.3 The influence of microorganisms on the conditioning of faecal pellets

As with the experiment that looked at the effects of temperature on the conditioning of faecal pellets three of the four treatments in experiment 1 showed a reduction in size between day 0 and day 3 of incubation. The only treatment that showed no decrease was treatment A, although this only showed a small degree of expansion of 2.5 %. After this initial decrease in surface area the treatments show two distinct patterns of processing. Treatments A and B show a continual increase in apparent surface area up to the end of the experiment, by which point the pellets have an apparent surface area 59 and 60 %

Figure 6.6; Experiment 1 on the influence of microorganisms on the conditioning of faecal pellets. Treatment A = faecal pellets in streamwater; B = faecal pellets in autoclaved streamwater; C = autoclaved faecal pellets in streamwater and D = autoclaved faecal pellets in autoclaved streamwater with added gentamicin. Error bars = 95% confidence intervals.

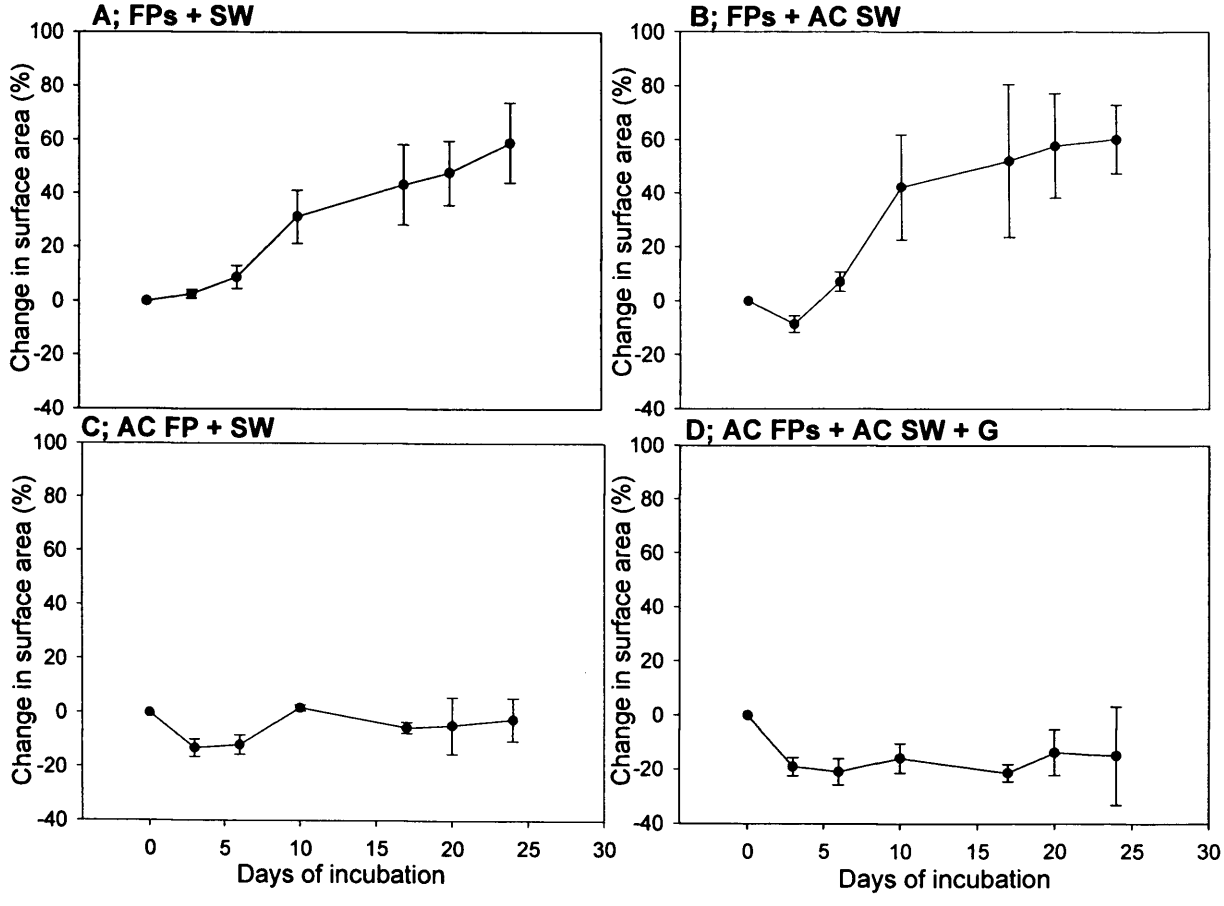


Table 6.2; Results of Mann Whitney tests showing significant differences in the increase in size of faecal pellets incubated under different treatments for experiment 1. Asterisks represent P values, * P = <0.05, ** P = <0.01, *** P = < 0.001.

B; FP + AC SW			
C; AC FP + SW	**	*	
D; AC FP +AC SW + G	**	**	*
	A; FP + SW	B; FP + AC SW	C; AC FP + SW

larger than at the start of the incubation. In contrast, treatments C and D have periods of increases in apparent surface areas, followed by decreases. By the end of the experiment treatment C was 2.5 % smaller and treatment D, 15 % smaller apparent surface areas than at the beginning (Figure 6.6). A Kruskal-Wallis test showed there to be highly significant differences between the treatments (P = < 0.001). Mann-Whitney tests showed treatments A and B were not significantly different from each other but were significantly different from treatments C and D. Treatments C and D were significantly different from each other (Table 6.2).

For experiment 2 only two of the treatments (C & D) showed a decrease in the surface area of the pellets on the first sampling occasion on day 4, the other two treatments showed an increase in surface area of 21 % for treatment A and 15 % for treatment B. Treatment A continually increases to day 18 when the faecal pellets have a 38 % larger surface area, from when the pellets are reduced in size to day 29 when the pellets are only 25 % larger. By the end of the treatment the pellets were 31 % larger than their original size. Treatment B also shows rises and falls in surface area after day 11 when it had risen to 30 %, surface area went as low as 20 % by day 29, the pellets finished 25 % larger than their original size.

Treatment C reached a maximum increase in surface area of 8 % by day 8, however, the surface area again fell below the original surface area by day 15. By the end of the experiment the pellets had a surface area 10 % lower than the original value. Treatment

D followed a similar pattern with increases in surface area followed by decreases. The surface area never increased in size above the initial surface area and the experiment finished 8 % below the original surface area.

The Kruskal-Wallis test showed highly significant differences in the % increases in surface area between the treatments (P = < 0.001). Mann-Whitney tests showed treatments A and B to be significantly different from each other but both A and B were highly significantly different from treatments C and D. Treatments C and D were not significantly different from each other (Table 6.3).

Figure 6.7; Experiment 2 on the influence of microorganisms on the conditioning of faecal pellets. Treatment A = faecal pellets in streamwater; B = faecal pellets in autoclaved streamwater; C = autoclaved faecal pellets in streamwater and D = autoclaved faecal pellets in autoclaved streamwater with added gentamicin. Error bars = 95% confidence intervals.

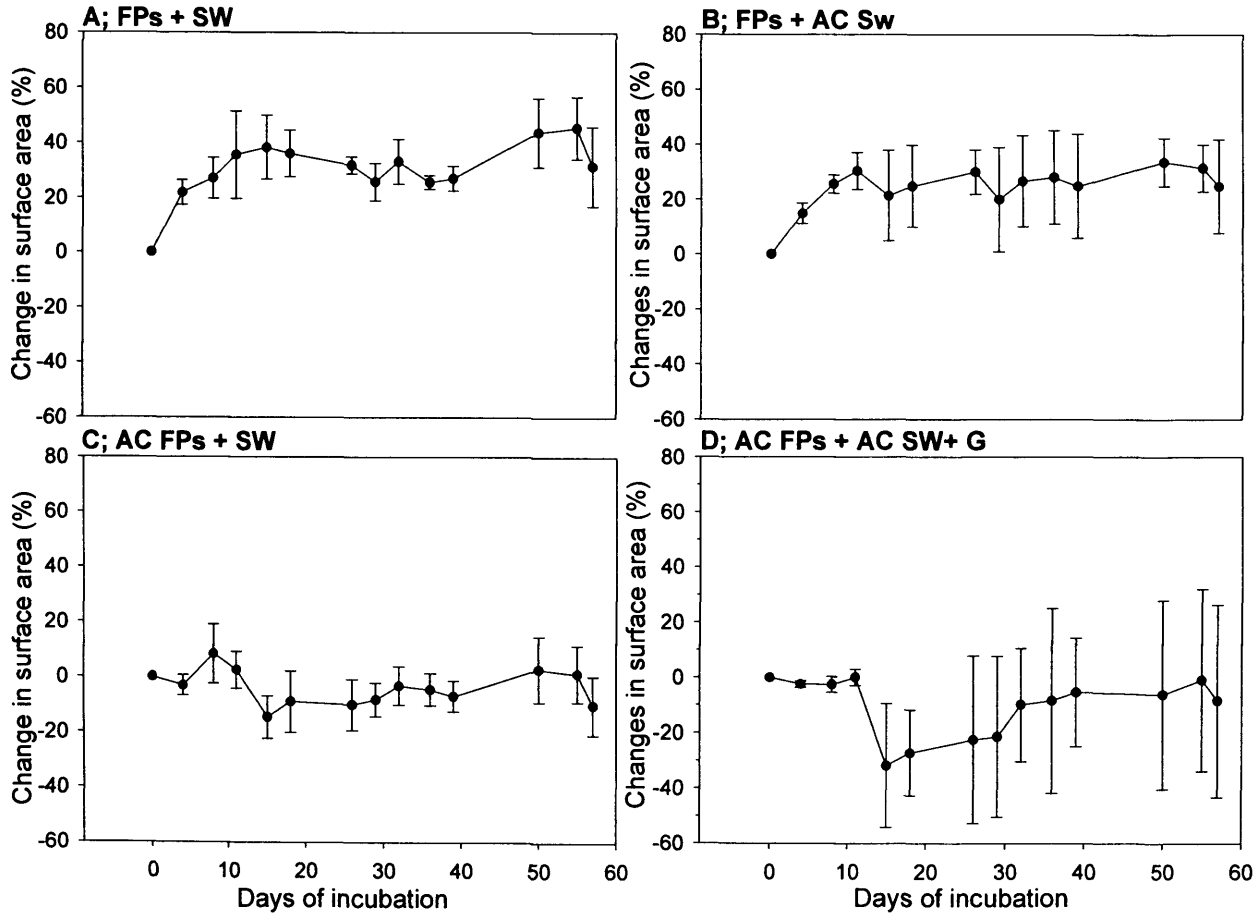


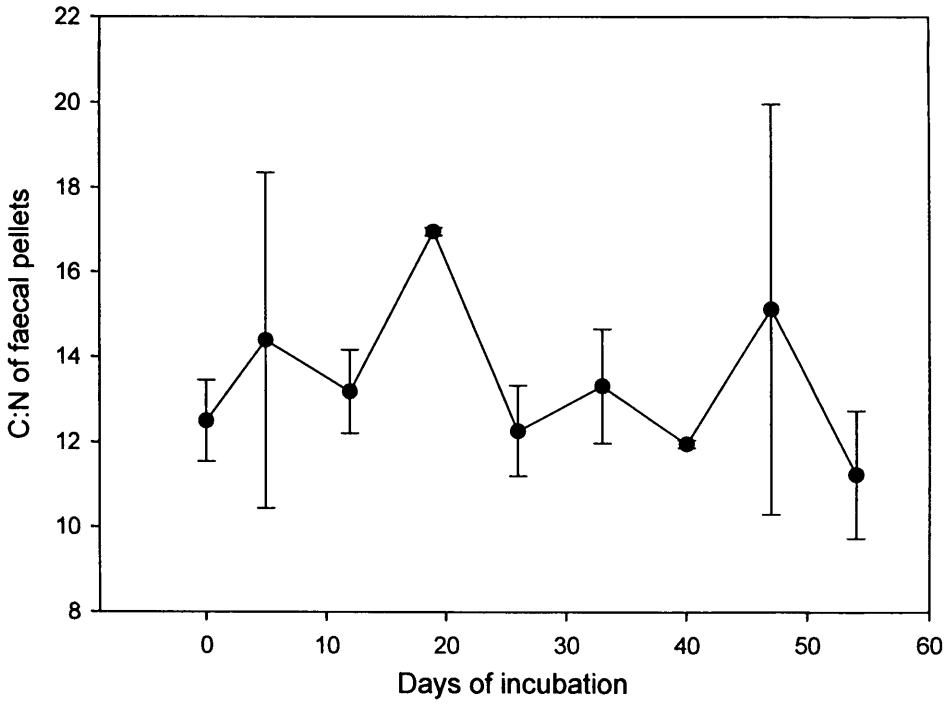
Table 6.3; Results of Mann Whitney tests showing significant differences in the increase in size of faecal pellets incubated under different treatments Experiment 2. Asterisks represent P values, * P = <0.05, ** P = <0.01, *** P = < 0.001.

B; FP + AC SW	*		
C; AC FP + SW	***	***	
D; AC FP +AC SW + G	***	***	
	A; FP + SW	B; FP + AC SW	C; AC FP + SW

6.3.4 Variations in C:N over time

The faecal pellets showed little variation in the C:N over time. The faecal pellets had an initial C:N value of 13, it reached a peak of 17 by day 19 and then reduced again to 12 by day 26. The values remained low until the end of the incubation with one rise on day 47 when the ratio rose to 15 (Figure 6.8).

Figure 6.8; Variations in the ratio of carbon to nitrogen over time. Error bars = 95% confidence intervals.



6.4 Discussion

The staining of the faecal pellet and gut material with Calcofluor showed that, although the blackflies clearly produce peritrophic membranes within the gut, these do not cover the faecal pellets after egestion. It is probable that the high numbers of faecal pellets produced by larvae, an average of 737 d^{-1} (Malmqvist et al., 2001), means that the larvae simply cannot produce enough membrane to cover each pellet. The staining of the pellets with the Alcian Blue showed pellets to contain large quantities of exopolymers. Disaggregation of the pellets showed the stain to have permeated into the pellet thus exopolymers are the dominant mechanism that binds blackfly faecal pellets together.

Temperature did not play an important role in the degradation of blackfly faecal pellets, with only the 4°C treatment being significantly different from the 10 and 15°C. Therefore it is expected that the degradation of faecal pellets deposited within chalk stream will not be influenced by variation in the ambient temperature conditions of the river.

Killing the microorganisms within the pellets had the effect of significantly reducing the degradation of the faecal pellets indicating that bacteria within the pellets play a key role in degradation following excretion into the environment, similar to that found for mussels (Fabiano et al., 1994). This suggests a different mechanism in streams for the degradation of faecal material, with an intrinsic microbial community, compared to the degradation of autochthonous and allochthonous material where the focus is on the invasion of micro-organisms from the stream environment into the material (Schlickeisen et al., 2003).

The pellets underwent stages when the apparent surface area of the material declined, particularly early in the incubation period. It is known that leafs exhibit extensive

decreases in mass, with initial leaching processes leading to a 25 % loss in mass of the material (Allan, 1995; Schlickeisen et al., 2003). These data relate to mass and not surface area but it would be expected that a similar loss of material from faecal pellets would lead to a decrease in the apparent surface area of the pellets. The loss of material coupled with the expansion of the pellets results in the pellets becoming less dense. The loss of density is deduced from a concomitant decrease in fall velocity as a result of increased porosity within the aggregation (Droppo et al., 1997).

The degradation of faecal pellets in the current study must be considered a minimum rate of degradation. Studies on zooplankton faecal pellets have compared the degradation of faecal pellets by microorganisms alone and microorganisms in conjunction with copepods. This demonstrated that the role played by the copepods was substantially greater than that played by the micro-organisms alone (Lampitt et al., 1990), a similar role may be attributable to macroinvertebrates and meiofauna in chalk streams.

The sedimentation of blackfly faecal pellets leads to the formation of organic deposits with an associated microbial community, much of this material is unlikely to have accumulated had it not been intercepted by the larvae. Processes encouraging the formation of organic deposits in other stream systems create 'hot-spots' of secondary production as the microbial community is consumed by other macroinvertebrates (Edwards & Meyer, 1990), blackfly-mediated biodeposits may represent a similar process in chalk streams. The ingestion and assimilation of stream microbes may then occur through either direct grazing of the microbes from the surface of the organic deposits (Edwards & Meyer, 1990; Pusch et al., 1998) or consumers may ingest the organic particles with the associated microbial community, but only assimilate the

microbial component thus egesting the organic substrate unaltered and ready for recolonisation by microorganisms (Kautsky & Evans, 1987; Fabiano et al., 1994; Pusch et al., 1998).

Blackfly larvae are non-selective feeders therefore the nutrient status of their faecal pellets is expected to mirror that of the ambient suspended seston. This is in contrast to organisms utilising selective feeding strategies which have faecal pellets of varying nutrient status dependent on the diet ingested (Mamelona & Pelletier, 2004). The C:N values of the blackfly larvae faecal pellets are high compared to those produced by marine bivalves, which are in the order of 7 – 9 (Giles & Pilditch, 2004), although values less than 3 have been recorded for the mussel *Aulacomya ater* (Stuart et al., 1982). The variation seen in marine bivalves was dependent on the quantity of phytoplankton in the diet, those pellets produced from bivalves feeding primarily on phytoplankton had the lowest C:N and were therefore of the highest nutritional value (Giles & Pilditch, 2004). In addition, bivalves are capable of selecting particles for ingesting so that faecal pellets will be expected to have enhanced nutritional quality in comparison to the bulk characteristics of the seston (Kautsky & Evans, 1987). Within marine systems phytoplankton has a C:N value of between 4 – 8, while bacteria are around 5.5, C:N ratios greater than 10 are characteristic of detritus (Kautsky & Evans, 1987). These values are low compared to values provided in the literature by Kendall *et al.* (2001) for freshwater systems. The value for POM was 9.4, periphyton was 14.6, submerged macrophytes was 22 while old woody material was > 80. In comparison to these data blackfly faecal pellets in the Frome and Piddle appear relatively nutritious.

7 General discussion

7.1 Blackfly larval faecal pellet dynamics in chalk streams

This study has determined the significance of blackfly faecal pellets within chalk streams and looked at the factors influencing the transport, storage and fate of this material. This expands on previous work looking at blackfly faecal pellets in the environment which have identified and described these pellets within chalk streams (Ladle & Griffiths, 1980); recorded its presence and abundance within other lotic environments (Wotton et al., 1998; Malmqvist et al., 2001; Malmqvist & Wotton, 2002); considered the implications of blackflies for particle flux through streams (Hershey et al., 1996; Wotton et al., 1996) and determined the dynamics of this material (Ladle et al., 1987; Wotton et al., 1998).

The feeding behaviour of blackfly larvae increases the size of particles found within the water column as larvae ingest particulate and dissolved organic matter, aggregate them into faecal pellets and excrete this material into the stream. As the larvae are capable of ingesting dissolved organic matter they increase the overall quantities of organic matter present in particulate form. Chalk streams are considered retentive systems capable of trapping and storing large quantities of fine particles (Berrie, 1992) and this was manifest in a number of ways. The Rivers Frome and Piddle contain very high numbers of blackfly faecal pellets in suspension. However, blackfly faecal pellets contributed relatively little as a proportion of suspended load compared to other systems studied. The volume of the faecal pellets sampled at different locations within the streams varied considerably. By far the smallest pellets were suspended in the water column, and this explains their minor contribution to suspended load. Pellets deposited on the substratum are typically much larger as the stream does not have the power to transport this material so they are deposited, this is indicative of the retentive nature of the system.

Additionally, peak faecal pellet abundance was seen in winter after peak larval abundance (Ladle et al., 1972; Ladle et al., 1977), indicating substantial storage of this material within the stream over the summer months before being exported from the system following increases in discharge and the dieback of macrophytes.

The storage of faecal pellets on the stream substratum is central to the role played by blackflies in organic matter dynamics within chalk stream. The material aggregated to form the pellets was originally transported in the water column prior to interception by the larvae and this creates a vertical flux of material from the water column to the substratum and hyporheic zone (Figure 7.1). The distance travelled by the pellet from excretion by the larvae to reaching the channel substratum, i.e. the strength of the vertical flux, is determined by a number of factors. The size of the pellet is critical, large pellets sink through the water column at a faster rate than small pellets; this helps explain the differences in size between pellets in suspension and those on the substratum described above. The density of pellets is also significant as denser pellets sink at a faster rate. The key determinant on the density of blackfly larvae faecal pellets is the density of the constituent particles that the larvae ingest. Larvae consuming a diet dominated by heavy, inorganic particles produce pellets that sink at a significantly faster rate than those ingesting relatively light organic particles.

Within chalk streams the stable, attenuated flow regimes ensure that diet plays a small role in determining sinking velocity as there are no 'flashy' flow events which would mobilise inorganic particles. Exceptions would be reaches with atypical particulate inputs to the stream such as high organic loads from impoundments or high mineral inputs caused by poaching of the banks by cattle. There is some seasonal variation in fall velocities when winter conditions alter the qualitative nature of the stream seston,

although these are of minor importance to particulate dynamics as larvae are overwintering during this period. The influence of diet is not a significant factor explaining faecal pellet transport in chalk streams. However it becomes significant when comparing faecal pellets between rivers with qualitative differences in their suspended load such as lake outlets (Wotton et al., 1998) or snow-melt rivers (Malmqvist et al., 2001).

The channel environment plays a key role in determining the fate of faecal pellets in chalk streams. The extensive macrophyte communities that are such a striking feature of these rivers exert a strong influence on the movement of particles and water. Early in the season, when *Ranunculus* is at peak biomass, the plant tissue occupies a large proportion of the channel and lowers water velocity through the reach, leading to enhanced trapping of the pellets within the plant stand (Figure 7.1). Once trapped within the stand the leaves and stems of the *Ranunculus* reduces the velocity of the flows as they enter the stand (Clarke, 2002) and the pellets are protected from resuspension and export from the reach. Pellets are also deposited in areas of retarded flow, particularly channel margins where friction between the bank and the water column causes a reduction in velocity. Here the standing stock of pellets is higher than that seen in *Ranunculus* stands, albeit with relatively low areal coverage. The magnitude and stability of these deposits is enhanced by riparian and marginal vegetation encroaching into the channel and providing shelter from the flows, similar to the effects provided by *Ranunculus*.

Later in the season encroachment of marginal vegetation constricts the area of open water in the channel, this is concurrent with a reduction in *Ranunculus* biomass post-flowering and leads to increased water velocity. The increased water velocity causes the

Ranunculus to reconfigure by compressing the above surface tissues to reduce drag upon the plant (Sand-Jensen, 2003). Reconfiguration deflects flows around and between stands while the compression of the tissues limits the interaction of water in the main channel with that in the plant. These changes to the macrophytes, and indirectly the flow, reduce the retention of pellets as the higher water velocities in the main channel increases the distance the pellets travel through the river before reaching the substratum. The higher water velocities will increase shear stresses at the water-substratum interface and above a critical shear stress deposition of pellets will cease totally (Cushing et al., 1993). Macrophytes are associated with extensive deposits of inorganic fine particles and these mechanically filter faecal pellets as water flows through the pores of the inorganic sediment. The presence of macrophytes within the channel alters flow paths and intensifies interactions between faecal pellets and inorganic sediments through zones of downwelling and upwelling as flows are transported through and around stands.

The interaction between the feeding activity of blackflies, the diet consumed and the chalk stream environment leads to extensive faecal pellet deposits that contribute to a significant proportion of the organic matter deposited within streams. A key difference between faecal pellet degradation and that of other detritus within streams is the microbial community bound within the pellet structure. Thus the degradation of faecal pellets does not have to undergo the initial stages of colonisation and invasion that plant tissue experiences (Schlickeisen et al., 2003). Additionally the provenance of much of the organic matter bound within the pellets will be from in-stream macrophytes and detritus from this source is processed at a faster rate than for that derived from allochthonous sources (Wanner & Pusch, 2001). The combination of organic rich, relatively labile, deposits with an abundant microbial community will produce sites of

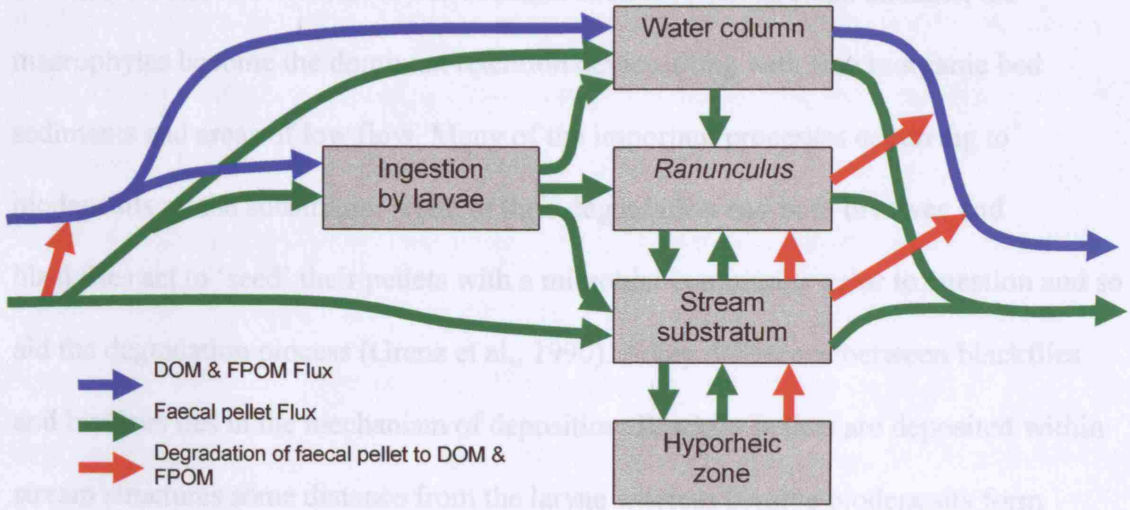
intense biogeochemical cycling. Studies on lowland macrophyte-dominated streams in New Zealand found that the low concentrations of dissolved oxygen found in the summer led to conditions promoting denitrification and methanogenesis (Wilcock & Croker, 2004) and there is evidence that similar processes occur in chalk streams (I. Sanders, pers. comm.). The microbial activity within the biodeposits will impact on the water chemistry through the release of soluble leachates to the water column and increased CO_2 from the microbial respiration of the sediments.

The degradation of faecal pellets is characterised by an increase in volume of the faecal pellets as the constituent particles begin to disaggregate. This dissaggregation process is likely to be similar to that seen in freshwater flocs where increased porosity, raises the water content of the particle so reducing density and modifying the transport characteristics of the aggregations (Droppo et al., 1997). Thus, a deposited pellet exposed to a constant shear stress will degrade and expand until its reduced density leads to the ambient shear stress exceeding the pellets critical shear stress so initiating resuspension into the water column and transport downstream. A pellet that has undergone degradation will travel an increased distance downstream than it would immediately post-excretion as the pellet's reduced density increases the time taken to settle through the water column thereby increasing displacement downstream. It follows that during events promoting the mass resuspension of faecal pellets from the stream substratum, such as the dieback or removal of macrophytes, older pellets will, on average, travel further downstream than fresher pellets.

The eventual fate of blackfly faecal pellets is export from the river to the sea, the retentive nature of chalk streams suggests that excretion from the larvae to export to sea will not occur in one step but as a series of transportation events interspaced with

periods of deposition. Figure 1 gives a schematic view of the movement of faecal pellets through a stream reach and shows the movement of faecal pellets between different zones within a stream. Pellets are excreted by the blackfly larvae and travel a distance downstream before deposition, either within a *Ranunculus* stand or onto the stream substratum, there they will begin to degrade eventually leading to resuspension followed by another period of transport downstream, and so on, until loss to the sea. During this process the pellets may be reingested by consumers, including other blackflies, and be repackaged as a fresh faecal pellet so restarting the process. The retention of organic matter by filter feeders allows enhanced utilisation of the retained nutrients by the stream community due to the increased residence time and has long been recognised as an important process within streams (Wallace et al., 1977). This study has shown chalk streams to represent a highly retentive system, with correspondingly short spiralling lengths, due to the interactions between the chalk hydrology, macrophytes and blackfly larvae which combine to retain particles within chalk streams through biological, physical and climatic processes.

Figure 7.1; Schematic diagram showing the transportation and fate of faecal pellets through a stream reach.



7.2 The wider context

The role played by blackflies in the modification and trapping of suspended particulate material is analogous to that of bivalves. Both ingest and aggregate particulate material into substantially larger discrete particles. These particles are modified to such a degree that their behaviour within the environment is altered dramatically from that of the original particles and from other particles in the environment. Both blackflies and bivalves produce pellets whose transport is primarily determined by the diet that the organism ingests (Giles & Pilditch, 2004); if an individual consumes a diet consisting of predominantly dense particles than the subsequent pellets will be relatively dense and so sink faster.

The production of biodeposits is an important consequence of pelagic-benthic coupling that is regulated by these organisms and represents an important transfer of material to the substratum altering conditions in the benthos (Vaughn et al., 2004). Within freshwater lotic systems the trapping and formation of biodeposits is dependent on retention devices. In upland streams particles are trapped by large stony substrates such as cobbles and boulders or by debris dams formed by large woody debris trapped in the stream (Wanner & Pusch, 2001). In lowland streams, such as chalk streams, the macrophytes become the dominant retention device along with fine inorganic bed sediments and areas of low flow. Many of the important processes occurring to biodeposits on the substratum relate to their degradation and both bivalves and blackflies act to 'seed' their pellets with a microbial community prior to egestion and so aid the degradation process (Grenz et al., 1990). A key difference between blackflies and bivalves lies in the mechanism of deposition. Blackfly pellets are deposited within stream structures some distance from the larvae whereas bivalve biodeposits form alongside the organisms, often as a result of reduced flow caused by the shell structure

of the bivalves (Ragnarsson & Raffaelli, 1999). The importance of the proximity of these deposits, either directly or indirectly, to the animal has yet to be determined.

The important role of bivalves in altering ecosystem processes is becoming increasingly understood (Strayer et al., 1999; Norkko et al., 2001; Vaughn et al., 2004). Their impacts on ecosystems have arguably been better studied than those of blackflies as a result of their commercial value and the invasive nature of some species. The effects of blackfly larvae on ecosystems have frequently focused on the role they play in the removal of material from the water column. This study demonstrates that they play a significant role in the transfer of particles to the substratum. The clear similarities in the functional role of bivalves and blackflies in aquatic environments indicates that a synthesis of the literature on bivalves into future research on the impacts of blackflies on stream processes will be productive.

As well as providing an example of invertebrate mediated transformation of fine particles the marine environment has abundant macrophyte communities that mirror those seen in chalk streams and perform many of the same functions. Seagrass communities are found in the littoral, photic zone of all continents, except Antarctica, at depths of between 0 – 30 m. These meadows, as they are commonly known, are sites of high primary production, important components of coastal food webs and regions with high rates of biogeochemical cycling (Duarte, 2002). Seagrass meadows have one of the highest rates of net primary production of any ecosystems in the world with values of >1000 g C m^{-2} yr^{-1} recorded (Borum & Sand-Jensen, 1996). Seagrasses along with mangroves and marsh plants are the three marine ecosystems dominated by angiosperms and contribute ~4 % to the total net primary production (NPP) of the oceans, however they store ~30 % of oceans NPP (Duarte & Cebrian, 1996) and this has

prompted significant research into the factors influencing the storage and processing of material within these systems. During the early 1930's disease nearly wiped out the seagrass communities of the North Atlantic and this correlated exactly with a period of dramatic coastal change in Denmark. Sheltered parts of the coast experienced sedimentation, while exposed areas suffered erosion (Christiansen et al., 1981), illustrating the influence of seagrass communities on the physical as well as biological processes.

Flow velocities are generally lower than those seen in chalk streams with recorded velocities in seagrass communities of between 2 – 10 cm s^{-1} (Gacia & Duarte, 2001). To cope with these flowing-water environments marine macrophytes have developed structural mechanisms in their above ground tissues and their growth forms are both flexible and extendible allowing the plants to reconfigure in order to reduce the drag acting on the plant (Koehl & Wainwright, 1977). This is a similar strategy to that employed by macrophytes occupying streams with high velocities (Sand-Jensen, 2003). The high above-surface biomass of seagrasses, in common with *Ranunculus*, alters the flow of water both around and within the canopy and this influences sediment stability. Flows within stands are reduced while those deflected over the plant are increased (Terrados & Duarte, 2000). Turbulence within the stands is reduced 2.5-fold compared to outside of the stands and this decreases the resuspension of sediments 3-fold in comparison to areas with no seagrass (Gacia & Duarte, 2001). Seagrasses reduce turbulence by transforming low frequency, large eddies at the seagrass-flow interface into high frequency, small eddies as they pass through the canopy (Madsen et al., 2001). The degree of resuspension was negatively related to canopy development while particle deposition was positively related to canopy cover, although the relationship was not strong (Gacia & Duarte, 2001). This finding corresponds with that by Wanner &

Pusch (2001) who found that freshwater macrophytes were relatively poor trappers of particles from the water column but were very efficient at storing the particles once they entered the stand. At certain times of the year, *Ranunculus* plants in chalk streams act to reduce the retention of particles within the channel. This suggests that resuspension, and not deposition, is the dominant control on sediment storage within macrophytes. Once deposited within a seagrass stand sediment stability is further enhanced by a dense network of rhizomes and roots which increases the cohesiveness of the sediments (Kenworthy et al., 1982).

Seagrasses cause qualitative changes in the characteristics of deposited particles by reducing mean particle size through the enhanced trapping of silts and clays and elevating the quantities of organic matter. The enhanced organic matter inputs to seagrass beds lead to increases in bacterial biomass which utilise the leached dissolved organic matter that is released early in the degradation process (Pedersen et al., 1999) and so creates conditions that support a high rate of biogeochemical processing (Kenworthy et al., 1982; Pedersen et al., 1999). Seagrass meadows act as a significant nitrogen sink as they take up nitrogen from the water column and convert it into biomass which, following senescence, is deposited onto the sediments beneath the seagrasses (Rysgaard et al., 1996). The rate of decomposition of seagrass tissue is less than production, leading to the accumulation of large quantities of organic matter within the meadows (Pedersen et al., 1999). The combined effects of plant biomass on flows, and therefore on particle dynamics, leads to extensive modification of the physico-chemical environment, corresponding to the role that *Ranunculus* plays in chalk streams. However, there are differences in the temporal nature of these deposits. Seagrasses are able to store large quantities of material over long time periods as they develop on scales up to centuries and thus steadily accumulate organic material (Borum

& Sand-Jensen, 1996). This contrasts to chalk streams macrophyte communities that undergo a relatively large and regular seasonal disturbance from winter frosts and scouring flows that removes much of the above surface macrophyte biomass and associated deposits each year.

Bivalves and seagrasses functionally perform the same processes as blackflies and *Ranunculus*. Within chalk streams blackflies are predominantly found on *Ranunculus* as these offer protection from predators, in contrast, bivalves are not reliant on seagrasses to provide a habitat in which to establish and the processes described above for the bivalve and seagrass refer to each community operating independently. Where bivalve aggregations are found in conjunction with seagrass meadows there can be significant interaction between the two. Populations of *Mytilus edulis*, at less than 20 % areal coverage, have beneficial effects on seagrasses, seen as increases in the size of the associated seagrass leaves. This is due to the input of bivalve biodeposits to the substratum which, when mineralised, release inorganic nutrients that fertilise the seagrasses through increases in the concentrations of ammonium and phosphate in the sediment porewater (Hemminga & Duarte, 2000). However, when bivalves reach high population densities they begin to negatively impact on seagrass communities. *Musculista senhousia* produces a matrix of sediment particles bound with byssus threads. The matrix forms a dense cover over the substratum and reduces seagrass rhizome growth rates (Reusch & Williams, 1998). Populations of *Mytilus edulis* can reach such high densities that they cover the substratum completely preventing seagrass growth and eventually excluding them completely (Hemminga & Duarte, 2000).

It would appear that in the marine environments the presence of bivalves is beneficial to the seagrasses whilst they remain at low population density, however as the population

densities increase then they exert a negative impact on the seagrasses. The sediment retaining properties of the seagrass beds is not expected to benefit the bivalves as this reduces the supply of particles for ingestion by the bivalves. This potentially negative effect may be offset by an increase in the nutrient status of particles within seagrass beds and possible protection from predators. In contrast, blackflies living within chalk streams benefit from their association with *Ranunculus*. They are protected from predators and due to the plants effects on stream flow are able feed in the areas of highest flows, and hence receive a high rate of particle supply from the passing current. The *Ranunculus* stands are also expected to benefit through the faecal pellets deposited within the stands which provide nutrients to the plants. The *Ranunculus* stands are not excluded by blackflies as seagrasses are by bivalves, although there may be some costs associated with a reduction in the photosynthetic surface areas as a result of larval attachment.

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